Modern Limnological and Paleolimnological Applications of Diatoms in Minnesota Lakes

A dissertation
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Dedication

I would like to dedicate this work to my wife Cristen Burge, my daughter Birdie Burge, and our soon to be born child. Primarily to Cristen for all of her unwavering support and patience while we grew our family, and I completed the following research here. In addition to working long difficult hours in an operating room to help support us, Cristen also provided wonderful recreational companionship in wilderness areas across North America and an intellectually stimulating partnership. I want to thank Cristen and our daughter Birdie for opening up parenthood as a new facet of life for me, full of the greatest joy and motivation I have ever experienced. I never knew life could be so great, thank you both for your patience during the completion of my dissertation.

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The academic road in diatom ecology is one filled full of supporting, friendly characters, part of my initial draw to the community. I have to thank my friends and J.C. Kingston Trio fellows Nicholas Schulte and Ian Bishop for all of our productive conversations, diatom sampling adventures, late nights in the lab, and fun musical reprieves. I would like to thank some of my mentors Sarah Spaulding, Marina Potapova, and Kalina Manoylov for your mentorship and inspirations. I would like to thank the friendly educational environment at the Iowa Lakeside Laboratory, especially Michael J. Lanoo, Matt Fairchild, Dianna Isder, and the Friends of the Lakeside Lab group, where I spent some very formative summers learning phycology and limnology. None of this would have even happened without my fantastic undergraduate mentors Drs. Katherine Larson, Ginny Adams, and Sally Entriken at the University of Central Arkansas. Of course, building upon the ecological connections I made during undergraduate studies I have to thank Travis D. Marsico and Billy G. Justus for their mentorship and friendships during my Master's of Science degree program at Arkansas State University. I would also like to thank the support of my colleagues at the St. Croix Watershed Research Station, Adam Heathcote and Joy Hobbs-Ramstack. Finally, I thank and really appreciate the guidance and patience provided by my committee members Euan Reavie, Daniel Engstrom and Teofil Nakov.

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Chapter 1

Introduction, Abstract, and Contributions

Science has intrigued me since I was a young child, Geography and Astronomy captured my wonder, and that amazement was heavily fostered by my mother who knew very little about the subject but got me connected with various programs, quizzed and encouraged me. Nature has always been one of my favorite places to recreate, so naturally when I was a teacher's assistant in high school I fell in love with Biology. Nearly a decade later, after working and traveling the earth to hike in some very beautiful places, I found myself sorting dead fish with graduate students during an invasive species eradication effort and I loved it. My wonderful undergraduate mentors pushed me to go onto graduate school, where in a Master's program and working for the US Geological Survey I first learned about diatoms.

Learning more about diatoms I enrolled in the Ecology and Systematics of Diatoms course at the Iowa Lakeside Laboratory. Here I met a wonderful group of researchers and began to learn more about a beautiful and complex group of mostly autotrophic organisms. These stramenopiles are unique in that they produce silica shells, called a frustule, which are highly ornate and provide species level identification based on their morphology. For a recently evolved organismal group, diatoms are highly speciated and diverse, inhabiting the ocean and freshwater habitats of all seven continents along a wide spectrum of environmental variables. The abundance of diatoms is apparent by their large

contribution to the global carbon/oxygen cycle and that they serve as a base for many aquatic food webs. In addition to the aforementioned benefits, diatoms have become a very useful tool for understanding change in aquatic ecosystems.

After using diatoms to develop indicators of wetland disturbance, describing new species, and contributing to diatoms.org, a peer-reviewed taxonomic reference for diatoms, I decided that I wanted to study the diatoms of Minnesota lakes with Dr. Mark B. Edlund. Over the course of the past 5 years and under the guidance of Dr. Edlund, I have conducted investigations that have contributed to lake management, expanded the known diversity of diatoms, and refined the methods for using DNA to assess diatom community composition in lakes of Minnesota. Here I briefly describe the research discussed in the following chapters with special attention to the questions posed and the significant contributions of my collaborators.

The following chapters represent a continuity of diatom research in Minnesota lakes. In Chapter 2, the study on Upper and Lower Red Lakes demonstrates the use of well-established paleolimnological proxies to assess eco-limnological change in a pair of large shallow lakes and inform management of the lakes by the Red Lake Tribal Department of Natural Resources and the Minnesota Pollution Control Agency. Highlighted here was the use of diatoms using traditional morphological and geochemical approaches to reconstructing limnological history. In Chapter 3, I present a review on resurrection ecology as a new tool in the paleolimnological tool belt. This chapter highlights diatoms as prime candidates for resurrection ecology studies and the use of sediment eDNA

to guide such studies. Chapter 4 uses the recommendations in Chapter 3 to leverage diatom microfossils and their DNA to examine the influence of 20th century dust deposition on productivity and community composition. This study highlights the first use of sediment DNA to characterize diatom assemblage changes in a North American lake, and furthermore highlights the beneficial uses of paired microfossil-DNA diatom proxies. In Chapter 5, I used sediment DNA to describe the genomic diversity of diatoms across lakes of Minnesota. The diatom diversity described here corresponds to the distribution of lake types across Minnesota that can be characterized by diatoms observed in light microscopy or by their DNA signatures. Furthermore, the paired use of diatom microfossils and sediment DNA showed similar limnological trends in the paleolimnological record of two lakes.

I would like to describe briefly each project with an emphasis on collaborators and their contributions. In Chapter 2 I collaborated with the Science Museum of Minnesota's St. Croix Watershed Research Station (SCWRS), the Minnesota Pollution Control Agency, and the Red Lake Band Department of Natural Resources to investigate the historical limnology of Upper and Lower Red Lakes. The Red Lakes have exceeded regional nutrient standards for the past 20 years; however, our paleolimnology evidence suggests that no significant major ecological changes have occurred in the last two centuries. Therefore, I suggest the 75th percentile of the modern water quality record to be used in establishing independent site-specific nutrient standards for both Upper and Lower Red Lakes. To complete this project, I recognize the assistance of the Red

Lake Band Department of Natural Resources (Shane Bowe), the Minnesota Pollution Control Agency (Jesse Anderson), Dr. Mark Edlund, and Dr. Adam Heathcote for their part in collection of surface water quality samples and sediment cores. Laboratory analysis for sediment core dating, geochemistry and diatom slide preparation was conducted by Dan Engstrom, Shawn Schottler, Alaina Fedie, Michelle Natarajan, and Erin Mortenson at the St. Croix Watershed Research Station, and the algae pigment and isotope analysis was conducted by Dr. Peter Leavitt's lab at the University of Regina.

In Chapter 3 I provided an extensive overview of diatoms as a model organism for resurrection ecology and paleolimnology, with an emphasis on the genus *Aulacoseira*. Here I emphasized the abundance of ecological knowledge and the commonality among species within this genus to produce resting stages. Dr. Edlund contributed to the overall structure of the paper and provided notes on diatom ecology. Dr. Dagmar Frisch contributed her experiences and expertise in resurrection ecology. The chapter is published in *Evolutionary Applications* as an open access article, where I retained the copyright to reproduce this article, including it here in my dissertation. The in-text citations reflect the style of the *Evolutionary Applications* journal and differ from Chapters 3-5; furthermore, several references have been updated since the submission of the article in 2017.

In Chapter 4 I investigated the ecological effects of dust-driven lake nutrient enrichment in Cedar Bog Lake, Minnesota. I observed proxies that suggest consistent low productivity and sedimentation rates for the 18th and 19th

centuries, but during the 20th century, the lake experienced a period of dust-driven nutrient enrichment which stimulated diatom growth. Diatom biogenic Si and diatom DNA mirrored increased organic matter suggesting a boost of productivity at the same time as an inorganic sediment influx. The diatom microfossil record showed improvements to lake water quality in recent decades, suggesting the pronounced dust-driven enrichment may have subsided, although productivity and sedimentation have not returned to historical levels. I would like to express thanks to Dr. Daniel Engstrom for his interpretations of the Cedar Bog Lake sediment history and the opportunity to work on the diatom microfossil, geochemical, and DNA data. I would also like to thank Joseph Craine at Jonah Ventures for writing the methods and conducting the DNA extraction, amplification, sequencing, and bioinformatics processing of the 23S amplicons for this project.

In Chapter 5 I leveraged subsamples from an existing SCWRS paleolimnological project across the state of Minnesota to detect occurrences of a cyanobacteria *Cylindrospermopsis*. Diatom DNA was extracted from sediment samples and analyzed to characterize community distributions in Minnesota lakes and paleo-lake sediments. I conducted preliminary investigations to match bulk sediment diatom DNA with some existing DNA reference libraries. I was able to detect environmental trends with diatom amplicon sequencing and observe assemblage changes over time in two sediment cores. In the Hill Lake sediment core, diatom microscope counts and amplicon sequence variants provided similar reconstructions in lake ecological history. This work highlights

using evolutionary history to determine taxonomy and the readiness of using amplicons to conduct biomonitoring in lakes of Minnesota. A great deal of gratitude is expressed to Dr. Adam Heathcote for sharing sediment subsamples and Hailey Sauer for her assistance collating sample metadata for these samples. These previously two mentioned people, in addition to Michelle Natarajan and Alaina Fedie, collected and processed sediment samples for this project. The DNA extraction, amplification, and sequencing were conducted at the University of Minnesota Genomics Center. I also express gratitude to Dr. Isabelle Domaizon of the of the French National Research Institute for Agriculture, Food, & Environment and Dr. Kathleen Stoof-Leichsenring of the Alfred Wegener Institute for their inspiration, encouragement, and advice in conducting this project.

Chapter 2

Managing the Red Lake Nation's and Minnesota's largest lake: Monitoring and paleolimnology support a site-specific standard for Upper and Lower Red Lakes (Red Lake Nation and Minnesota, USA)

Abstract

The challenge of lake management increases as lakes are subjected to influences such as climate change, aquatic invasive species, and nutrient enrichment. Regionally tailored assessment standards are designed to make assessing the ecosystem health of many lakes easier. Management strategies should incorporate historical knowledge, reference conditions, predictive and retrospective models, and assessment of downstream effects. In Minnesota when lakes exceed state water quality standards, then either remediation plans are implemented, or the body of water can be assessed for a site-specific standard. Upper Red Lake and Lower Red Lake, Minnesota, USA have experienced increased cyanobacteria bloom occurrences as well as routinely exceeding the regional nutrient criteria for total phosphorus and chlorophyll-a. The last 20 years of water quality monitoring data show a stable recent history with no significant trends. Historical changes in land-use and biogeochemical evidence from paleolimnology were used to investigate the limnological history of these two large shallow lakes in order to determine if a site-specific nutrient standard should be recommended. Biogeochemical evidence (sedimentation, phosphorus fractionation, biogenic silica, diatom community analysis, and algae pigment data) from 10 sediment cores revealed complex dynamics within these

large shallow basins and a subtle increase in primary production over the 200-year reconstruction of the lakes. While a subtle productivity increase was observed, diatom-inferred phosphorus showed no significant changes in the sediment cores and predicted total phosphorus values that were within the range of modern measured values. The evidence here indicates little change in water quality in the Red Lakes, and we recommend a site-specific nutrient standard for managing this large shallow northern lake.

2.1 Introduction

Lake ecosystems provide highly valued services such as clean drinking water, recreation, transportation, fisheries, and biotic refugia. The quality of lakes can impact recreational revenue (Keeler et al. 2015) or property values of homes (Reynaud and Lanzanova 2017). Management for lake quality is complicated by watershed-land-use, non-native species invasion, cyanobacteria toxins, degradation of water quality, and climate change. For example, nutrient loading from landscape changes or the warming of lakes can promote cyanobacteria growth and higher concentration of their toxins that are harmaful to humans, livestock, and domestic pets (Paerl & Huisman 2008, Wagner & Adrian 2009, Kosten et al. 2012). Lakes even in pristine watersheds can be altered by atmospheric deposition of nutrients (Spaulding et al. 2015) or increasing global temperatures (Woolway et al. 2019). Crucial to sustainable management of these aquatic ecosystems, lake monitoring and knowledge of historical influences are fundamental to understanding the biogeochemical trajectory of lakes (U.S. Environmental Protection Agency 2000, European Union 2000).

With recognition for the value of lakes and the complications of intensively surveying each lake, standard measurable criteria are established to facilitate broad scale monitoring. The U.S. Environmental Protection Agency (USEPA) initially recommended thresholds to govern lakes based upon the limiting nutrients nitrogen and phosphorus (USEPA 1976). The USEPA recognized that there is geographic variability to the natural reference conditions for lakes and subsequently divided management efforts by ecoregions (USEPA 2000). Within each of these regions, management standards are established through a protocol, incorporating historical knowledge, reference conditions, models, scientific data interpretation, and assessing downstream effects. Monitored biogeochemical constituents include total phosphorus (TP), total nitrogen (TN), chlorophyll-a (chl-a), and Secchi depth. Following these guidelines, several states (e.g., Ohio, Minnesota, Vermont) have further refined lake monitoring programs (Heiskary and Wilson 2008, Skalski and Anderson 2010, Vermont 2015).

In Minnesota, a “weight of evidence” approach was taken to develop lake nutrient criteria (Heiskary and Wilson 2008). Lakes occurring across Minnesota vary by ecoregion, trophic state, depth, watershed influences (e.g. land-use, vegetation, geology), and lake history, which cumulatively result in distinct lake ecologies (Eddy 1938, Moyle 1956, Moyle 1945, Bright 1968, Burgam 1983, Heiskary et al. 1987). Recognizing the regional diversity of Minnesota’s lake types (Heiskary et al. 1987), a criterion was initially established to evaluate phosphorus concentrations and its impacts on lake conditions and users and

watershed mass-balance (Heiskary and Walker 1988). Following the revised USEPA (2000) guidelines for lake monitoring and building upon extensive Minnesota lake research, Heiskary and Wilson (2008) established lake condition criteria for three Minnesota ecoregions for the explanatory variable total phosphorus, and response variables, chlorophyll-a and Secchi depth. They observed the North Central Hardwood Forests and Western Corn Belt Plains ecoregions' shallow polymictic lakes (maximum depth <8 m) were distinctly different from deeper dimictic lakes (maximum depth > 10 m). Shallow lakes in these regions tended to frequently mix, having higher TP, chl-a, and reduced Secchi depths. For these shallow lakes, hypereutrophic lakes have high nutrients and algae dominance compared to lower trophic states where the lakes are macrophyte-dominated with less nutrients (Moss et al. 1996, Moss 1998). While the exact tipping point varies by lake, the eutrophic TP range (60-90 μg/L) has been shown to coincide with macrophyte decline and algae bloom increases (Heiskary and Lindon 2005, Heiskary and Wilson 2008). Furthermore, paleolimnological evidence comparing pre- and post-European settlement diatom-inferred phosphorus showed a nutrient increase in shallow lakes with disturbed watersheds outside of the Northern Lakes and Forests ecoregion (Heiskary and Swain 2002, Ramstack et al. 2003, Heiskary et al. 2003, Heiskary et al. 2004, Ramstack et al. 2004, Heiskary and Lindon 2005).

The aforementioned paleolimnology studies were a crucial line of evidence for determining historical lake conditions and establishing nutrient criteria where anthropogenic disturbance had altered the modern ecology,

especially for the shallow lakes (Heiskary and Wilson 2008). Paleolimnology relies on a wide variety of biotic and geochemical parameters as proxies for ecological dynamics that can be measured within a conformable, dated sediment core to interpret historical limnological changes (Cohen 2003, Last and Smol 2002). Paleolimnology has revealed that shallow lakes are prone to perturbations from changes in hydrology (Bradbury et al. 2004, Yang et al. 2018) and nutrient enrichment (Wang et al. 1988, Spaulding et al. 2015). The sediment record of the large shallow Lake Markermeer showed the polymictic nature of the lake, that sediment resuspension was frequent, and sediment accumulation occurred only in the deepest parts of the lake (Kelderman et al. 2012). In a survey of 32 productive Danish lakes, surface sediment phosphorus was found to be correlated with the external phosphorus load and lake sediment iron (Søndergaard et al. 1996). Furthermore, while the surface sediment iron-bound phosphorus was found to be mobile, it was also found to be permanently buried downcore. Nõges and Kisand (1999) found a horizontal distribution of phosphorus fractions in a large shallow lake, where mineral-bound phosphorus was the prevalent fraction in erosive, non-depositional areas. In addition to measuring sediment phosphorus, diatoms have been a useful paleo-proxy for detecting environmental change and estimating historical total phosphorus especially in Minnesota shallow lakes (Ramstack et al. 2003, Ramstack et al 2004). Studies in Minnesota are a key example of how paleolimnology can be used to inform nutrient standards in lakes where anthropogenic disturbances

have been present for over a century (Heiskary and Wilson 2008, Bennion et al. 2011).

Paleolimnology of a large shallow lake, Lake of the Woods, was used to determine the trajectory of lake ecology and the historical frequency of cyanobacteria bloom conditions (Reavie et al. 2017, Anderson et. al 2017). Reavie et al. (2017) used paleolimnological evidence to trace Lake of the Woods ecology before, during, and after nutrient perturbation in the mid 20th century. Multiple sediment cores indicated a community shift over the 20th century trending to diatoms with a higher total phosphorus optimum (Reavie et al. 2017). Over the last 30 years a second reorganization was observed with a shift to eutrophic planktonic diatoms. Working with the same sediments, Edlund et al. (2017) used paleolimnological evidence to inform models of phosphorus dynamics within Lake of the Woods. They found the legacy effects of mid-century phosphorus enrichment to now be minimal and therefore the unseen ecological trajectory of the lake must be attributed to other factors such as climate change. These studies highlight the utility of paleolimnology to generate multiple lines of evidence in the absence of historical monitoring data to inform lake management decisions (Anderson et al. 2017).

In recent years, cyanobacteria blooms have been observed especially in Upper Red Lake, Minnesota, USA. Upper Red Lake and Lower Red Lake are two large, shallow (<10 m) connected basins that are remnants of glacial Lake Agassiz (Wright et al. 1992). The lakes are managed by the Red Lake Band of Chippewa in partnership with the State of Minnesota, the latter responsible for

management of the far eastern portion of Upper Red Lake. Watersheds of the lakes are at the crossroads of 3 Minnesota ecoregions, with mostly northern peatlands draining into Upper Red Lake and a mosaic of hardwood and coniferous forested watershed, draining into Lower Red Lake. Upper Red Lake drains into Lower Red Lake, which have 10.8-year and 12.7-year residence times respectively (Anderson 2017). While monitored Secchi depth (m), chlorophyll-a ($\mu g\ L^{-1}$), and total phosphorus ($\mu g\ L^{-1}$) have exceeded Minnesota's state guidelines for the Northern Lakes and Forest ecoregion, no significant trends for these parameters have been observed in over 20 years of monitoring (Anderson 2017). Dollinger et al. (2017) found that within the relatively small, undisturbed watershed of the Red Lakes, about 1/3 of the tributaries and lakes were impaired for aquatic life or recreational use due to natural factors such as excessive sediment or high organic matter. Significant events in the Red Lake's history include logging in the watershed during the beginning of the 20th century (Albrecht and Thomas 1977), widespread attempted drainage of the northern peatlands during the 1910s to 1930s (Volstead act of 1906), damming of Lower Red Lake in 1931, and collapse and recovery of the walleye fishery at the turn of the 21st century (Pereira et al. 1992, Gangl and Pereira 2011). Regional climate trends indicate increasing minimum temperatures and decreasing average wind speeds (Reavie et al. 2017). In the absence of a longer monitoring record, a paleo-limnological investigation was warranted to ascertain the trajectory of the Upper and Lower Red Lake's ecology and to place current conditions in context with historical lake conditions (Reavie et al. 2017, Yang et al. 2018).

2.2 Methods

2.2.1 *Coring methods*

To determine the spatial variability in sediment deposition, three piston-cores were collected from each Red Lake basin along east-west transects during March of 2016. Four additional cores were collected in the western portion of Upper Red Lake during February of 2018 (Figure 2.1, Table 2.1). Sediment cores were collected with a 6.5 cm diameter polycarbonate tube using a piston and drive rod system (Wright 1991) with recovery ranging 69 cm to 102 cm (Table 2.1). Recovered sediment cores were maintained in a vertical position and Zorbitrol was added in order to stabilize the sediment-water interface during transport. All sediment cores were subsampled in 0.5-cm increments for the top 10 cm and 1-cm increments below that, except core UC1B which was sectioned in 1-cm increments in its entirety. Coring methods are discussed in greater detail in Reavie et al. (2017), Edlund et al. (2017), and Burge et al. (2018).

2.2.2 *Water Quality*

Spatial and temporal trends in total phosphorus (*μ*g/L), chlorophyl-a (*μ*g/L), and Secchi depth (m) were explored using monitoring data provided by the Red Lake Department of Natural Resources. During the open water season, the Red Lake DNR sampled water quality samples at five stations on east to west transects in each basin monthly since 1999. The water chemistry data were subset for the growing season May to October when samples were available. One-way ANOVA was conducted to detect within basin differences, and, to

investigate between basin differences, values from each basin were compiled and analyzed in a second one-way ANOVA.

2.2.3 *Dating and Geochemistry*

The sediment cores were analyzed for ^{210}Pb activity to determine age and sediment accumulation rates for the past 150 to 200 years. Lead-210 activity was measured from its daughter product, ^{210}Po, which is considered to be in secular equilibrium with the parent isotope. Aliquots of freeze-dried sediment were spiked with a known quantity of ^{209}Po as an internal yield tracer and the isotopes distilled at 550°C after treatment with concentrated HCl. Polonium isotopes were then directly plated onto silver planchets from a 0.5 N HCl solution. Activity was measured for 1-3 x 10^5 s using an Ortec alpha spectrometry system. Supported ^{210}Pb was estimated by mean activity in the lowest core samples and subtracted from upcore activity to calculate unsupported ^{210}Pb. Core dates and sedimentation rates were calculated using the constant rate of supply model (Appleby and Oldfield 1978, Appleby 2001). Dating and sedimentation errors represented first-order propagation of counting uncertainty (Binford 1990).

Bulk-density (dry mass per volume of fresh sediment), water content, organic content, and carbonate content of sediments were determined by standard loss-on-ignition techniques (Dean 1974). Weighed sediment subsamples were dried at 105°C for 24 hr to determine water content and dry bulk density, then heated at 550°C and 1000°C to calculate organic and carbonate content from post-ignition weight loss, respectively. These data were used in combination with ^{210}Pb dating to calculate sedimentation rates as dry

mass accumulation rates (DMAR; g cm^{-2} yr^{-1}) for each core and its sediment constituents.

Biogenic silica (BSi), a proxy for historical diatom and chrysophyte algal productivity, was measured using weighed subsamples (30 mg) from the cores, which were digested for BSi analysis using 40 ml of 1% (w/v) Na_2CO_3 solution heated at 85°C in a reciprocating water bath for five hours (DeMaster 1979, Conley and Schelske 2001). A 0.5 g aliquot of supernatant was removed from each sample at 3, 4, and 5 hr. After cooling and neutralization with 4.5 g of 0.021N HCl solution, dissolved silica was measured colorimetrically on a Unity Scientific SmartChem 170 discrete analyzer as molybdate reactive silica (SmartChem 2012a).

Sediment phosphorus fractions were analyzed following the sequential extraction procedures in Engstrom (2005), Engstrom and Wright (1984), Psenner and Puckso (2008), and Kopácek et al. (2005). Extracts were analyzed colorimetrically on a Unity Scientific SmartChem 170 discrete analyzer using methods described by SmartChem (2012b). Measured sediment P concentrations were also converted to flux using bulk sedimentation rates in each core. In addition to total phosphorus in cores, sediment fractions include the refractory forms *Mineral-bound P*, *Recalcitrant Organic-P*, *Al-bound P* and the labile or readily exchangeable forms of *Fe-bound*, *labile Organic-P*, and *loosely-bound P*.

2.2.4 *Pigment and Isotope Analyses*

Algal pigment analyses were performed on 15 sections each from cores LC3B and UC1B. Carotenoids, chlorophylls, and derivatives were extracted (4°C, dark, N_2) from freeze-dried sediments according to Leavitt and Hodgson (2001), measured on a Hewlett-Packard model 1050 high performance liquid chromatography system, and are reported relative to total organic carbon (TOC; Hall et al. 1999). Stable C and N isotope ratios and elemental composition were determined on unacidified whole sediment samples from core LC3B using a ThermoQuest (F-MAT) Delta^{PLUS} XL isotope ratio mass spectrometer equipped with a continuous flow (Con Flo II) unit, an automated Carlo Erba elemental analyzer as an inlet device, and following standard procedures of Savage et al. (2004). Stable N ($\delta^{15}N$) and C ($\delta^{13}C$) isotopic compositions were expressed in the conventional δ-notation in units of per mil (‰) deviation from atmospheric N_2 and an organic C standard which had been calibrated previously against authentic Vienna Pee Dee Belemnite. Sample reproducibility was <0.25 ‰ and <0.10 ‰ for $\delta^{15}N$ and $\delta^{13}C$ determinations, respectively.

2.2.5 *Diatom and Numerical Analyses*

Diatoms were prepared by placing approximately 30 mg freeze dried core material in a 50 ml polycarbonate centrifuge tube and adding 2-5 drops of 10% v/v HCl solution to dissolve carbonates. Organic material was subsequently oxidized by adding 10 ml of 30% H_2O_2 and heating for 3 hr in an 85°C water bath. After cooling, the samples were centrifuged and rinsed six times with deionized water to remove oxidation byproducts. Material was then transferred to Battarbee chambers where the cleaned material dried onto coverslips (Battarbee

1973). Coverslips were permanently attached to microscope slides using Zrax mounting medium (Ramstack et al. 2008). Diatoms were identified along measured random transects to the lowest taxonomic level under 1000-1250X magnification (full immersion optics of NA>1.3). Six hundred valves were enumerated in each sample using the voucher flora method (Bishop et al. 2017). Valve density, a measure of overall diatom abundance, was calculated using a modification to the equation provided by Scherer (1994) where the area of the bottom of the beaker was replaced by the area of the Battarbee settling chamber. The equation $T = (NB/AF)/M$ was used where T is the number of microfossils per unit mass, N is the total number of microfossils counted, B is the bottom area of the Battarbee chamber (mm^2), A is the area per field of view (mm^2), F is the length of the transect (mm), and M is the mass of the sample (g). Identification of diatoms relied on floras and monographs such as Hustedt (1927-1966, 1930), Patrick and Reimer (1966, 1975), Krammer and Lange-Bertalot (1986-1991), Reavie and Smol (1998), Camburn and Charles (2000), Fallu et al. (2000), and Reavie and Kireta (2015). Diatom counts were converted to percentage by species or taxon; abundances are reported relative to total diatom counts in each sample.

To determine community similarity and changes within and among cores, Non-metric Multi-Dimensional Scaling (NMDS) was implemented in the *vegan* package in R (R Core Team 2014, Oksanen et al. 2013). Square root transformation was applied to the relative proportion diatom data using the Hellinger method (Legendre and Gallagher 2001). Transformed data were then

converted into a distance matrix using Euclidian measures and the NMDS was performed using Bray-Curtis dissimilarity. Stratigraphy of predominant diatoms (species with greater than or equal to 3% relative abundance in one or more core depths) was plotted against core date using the *Rioja* R package (Juggins and Juggins 2019). Temporal relationships among the dominant diatom communities within each sediment core were explored using the Euclidian distance matrices in a constrained cluster analysis (CONISS). In the *Rioja* R package, significant cluster groups were determined using the broken stick model (MacArthur 1957).

Downcore diatom communities were also used to reconstruct historical epilimnetic total phosphorus levels. A transfer function for reconstructing historical logTP was developed earlier based on the relationship between modern diatom communities and modern environmental variables in 89 Minnesota lakes (Ramstack et al*.* 2003, Edlund and Ramstack 2006) using weighted averaging (WA) regression with inverse deshrinking and bootstrap error estimation (Juggins 2003, Juggins and Juggins 2019). The strength of the transfer function was evaluated by calculating the squared correlation coefficient (r^2=0.83) and the root mean square error (RMSE=0.181) between the observed logTP with the model estimates of logTP for all samples. Bootstrapping was used in model validation to provide a more realistic error estimate (RMSEP, the root mean square error of prediction=0.209 logTP units) because the same data are used to both generate and test the WA model (Fritz et al*.* 1991). Reconstructed estimates of logTP (diatom-inferred TP, or DI-TP) for each downcore sample were determined by taking the logTP optimum of each species, weighting it by its

abundance in that sample, and determining the average of the combined weighted species optima. Data are modeled as logTP values but presented as back-transformed values of TP in μg/l or ppb.

To evaluate the strength of the reconstruction we determine the amount of variance in the diatom data that can be accounted for by the TP reconstruction. This is calculated by the variance explained by the first axis of an ordination of the sediment assemblages constrained to diatom- inferred TP, divided by the variation explained by an unconstrained ordination of the sediment assemblages ($\lambda r/ \lambda p$). A maximum $\lambda r/ \lambda p$ value of 1.0 would mean that TP was the best explanatory variable of diatom community change (Juggins et al. 2013).

Using mean annual growing season TP data derived earlier from modern water quality data, the historical DI-TP was compared with modern measured TP values. Furthermore, modern (>1950) and historical (<1900) DI-TP values were parsed and averaged by basin based upon any significant change detected through the cluster analysis of the diatom community. Using ANOVA, the six pools of TP or DI-TP data were examined for significant differences to infer any changes in Upper and Lower Red Lake.

2.3 Results

2.3.1 *Sediments and Dating*

Unsupported ^{210}Pb ranged from 0.084 pCi/g to 21.20 pCi/g and generally followed a monotonic downcore trend (Figure 2.2). The ^{137}Cs profiles also showed single peaks corresponding with the mid-20th century, affirming conformable sedimentation and the dating profiles for the Lower Red Lake cores

and UC1 (Supplemental figure 2.1, Supplemental figure 2.2). All sediment cores recorded some flattening of the unsupported ^{210}Pb profile over the uppermost ~10 cm. Levels of supported ^{210}Pb were reached at a wide variety of depths ranging from 11 cm to 31 cm. Sediment core UC2A recorded an anomalous spike in unsupported ^{210}Pb between 20 cm and 30 cm and core UC3 had low and variable unsupported ^{210}Pb record that was limited to the top 7 cm (Supplemental Figure 2.2). The ^{210}Pb dating records indicate areas of central and eastern Upper Red Lake have unconformable sedimentation. Furthermore, ^{137}Cs and ^{210}Pb in the UC2A and UC3 had asynchronous fluctuations and multiple peaks and indicated non-conformable sediment accumulation (Supplemental figure 2.2) and were rejected from most further analyses.

Dry mass accumulation (DMAR) followed a general increasing trend across all cores ranging from 0.0025 g cm^{-2} yr^{-1}to 0.0968 g cm^{-2} yr^{-1}. The sediment cores LC1, LC2, LC3B, UC1B, and UC4 had low but steadily increasing sediment rates (<0.027 g cm^{-2} yr^{-1}), whereas UC1, UC2A, and UC3 displayed the highest sedimentation rates with variable trends (Figure 2.2). The cores UC1, UC2A, and UC3 were all marked down core by a high deposition event estimated (> 0.075 g cm^{-2} yr^{-1}) at 25 cm, 33 cm, and 8 cm respectively. Given the highly variable nature of UC1, UC2A, and UC3, unsupported ^{210}Pb were checked using ^{137}Cs. A ^{137}Cs peak for UC1 occurred between 13 cm to 14 cm which corresponded with the ^{210}Pb years estimated to 1968 to 1962 respectively. All cores from Lower Red Lake and UC1, UC1B, and UC4 demonstrated

conformable sedimentation and estimated dates were modeled back to at least the late 19th century.

2.3.2 *Geochemistry*

The Lower Red Lake cores were composed of 35% to 45% organic matter by weight, higher than the Upper Red Lake cores, which were usually between 20% and 30% organic matter (Figure 2.3). The opposite was true for inorganic matter with Upper Red Lake sediments composed of 55% to 65% inorganics and Lower Red Lake sediments ranging between 40% and 55%. Upper Red Lake also had 10% to 20% $CaCO_3$, about 5% more carbonates than Lower Red Lake. All cores showed gradual decreases in the percentage of inorganic sediments and conversely increases in the percentage of organic sediments up core, marked by a spike in organic matter at the tops of the cores. Dry mass accumulation rates (DMAR) gradually increased over the 20th century in all cores. Core UC1 showed a marked accumulation event of 0.097 g/cm^2 yr^{-1} dated to 1914, composed of a 15% increase of inorganic sediments.

All of the cores show increases in sediment phosphorus concentration and flux between the oldest and most recent sediments. Upper Red Lake had lower sediment total P concentrations, 0.8 mg P/g, compared to Lower Red Lake which was on average 1.4 mg P/g (Figure 2.4). In Lower Red Lake cores, the recalcitrant organic P fraction is greatest. In Upper Red Lake, the recalcitrant organic-P and mineral bound-P fractions have similar concentrations, 0.25 mg P/g and 0.29 mg P/g respectively. The concentration of mineral bound-P (max: 0.44 mg P/g, min: 0.08 mg P/g) decreases over time while the other fractions –

recalcitrant organic-P (max: 1.2 mg P/g, min: 0.1 mg P/g), labile organic-P (max: 0.37 mg P/g, min: 0.04 mg P/g), Al-bound-P (max: 0.44 mg P/g, min: 0.08 mg P/g), Fe-bound-P (max: 0.56 mg P/g, min: 0.03 mg P/g), and loosely bound-P (max: 0.04 mg P/g, min: 0.00 mg P/g) – increase in concentration upcore.

Monitored total phosphorus showed no significant spatial differences within each basin (Table 2.2, Figure 2.5), however Upper Red Lake (45.9 *μ*g/L) had significantly higher water column TP values compared to Lower Red Lake (37.2 *μ*g/L) ($P < 0.001$). Chlorophyll-a was significantly higher ($P< 0.001$) in Upper Red Lake compared to Lower Red Lake, 16.3 *μ*g/L and 10.4 *μ*g/L respectively. Chlorophyll-a corrected for pheophytin was higher in Upper Red Lake compared to Lower Red Lake, 12.9 *μ*g/L and 11.7 *μ*g/L respectively, although the difference was marginally significant ($P=0.06$). Average Secchi depth was significantly lower ($P < 0.001$) in Upper Red Lake compared to Lower Red Lake, 0.7 m and 1.1 m respectively.

3.3.3 *Pigments & Stable Isotopes*

Algal pigment records were recovered from cores LC3B and UC1B. Pigment concentrations from both cores generally showed a sharp increase at the core top (Figure 2.6). Fucoxanthin (diatoms and some dinoflagellates) was low and remained nominal throughout the cores until the most recent decades where it began a sharp increase to 50.4 nmol g C and 73.0 nmol g C for LC3B and UC1B respectively. Diatoxanthin (diatoms, chrysophytes, and dinoflagellates) ranged from 7.1 nmol g C to 44.7 nmol g C, with a slight increase in UC1B over the 20th century and no clear trends in LCB3. Diadinoxanthin

(diatoms, chrysophytes, dinoflagellates) and alloxanthin (cryptophytes) showed a gradual increase over the 20th century. Canthaxanthin (Nostocales) was higher in LC3B (35.5 nmol g C on average), whereas UC1B was lower (21.3 nmol g C on average). Aphanizophyll (Nostocales, *Aphanizomenon*) was absent in LC3B and UC1B until the beginning of the 21st century where it peaked at 5.1 nmol g C and 30.5 nmol g C, respectively. Pheophytin-B and β-carotene showed small increases at the beginning of the 20th century and variable trends until a spike at the 21st century. Chlorophyll-a showed a slow gradual increasing trend in both cores over the 20th century followed by a sharp peak at the top of the core. Chlorophyll-b followed a similar trend to chlorophyll-a except UC1B had no detections of chlorophyll-b throughout the 20th century. Echinone and lutein show no clear trends over the 20th century in the Red Lakes. Carbon (C) and Nitrogen (N) isotope geochemistry from core LC3B revealed decreasing trends in the $\delta^{13}C$ and C:N, while the percentages of C and N both increased toward the top of the core (Supplemental Figure 2.3). Both the isotopes and pigments indicated good preservation of sediment pigments and productivity signals. The pigment data did not indicate any community shifts between algal groups, whereas the isotope data indicated gradual increases in productivity.

2.3.4 *Diatoms and inferred historical TP*

Historical diatom productivity was examined using biogenic silica (BSi) concentration and flux and a count-based abundance of diatom microfossils. Percent BSi was less in Upper Red Lake compared to Lower Red Lake, and both lakes exhibited a gradual trend of increased concentration over the 20th century

suggesting increasing productivity (Figure 2.7, min: 2.2 mg g^{-1} SiO_2, 9.9 max: mg g^{-1} SiO_2). The BSi flux similarly supports slowly increasing productivity in the 20th century (Figure 2.7, min: 0.1 mg cm^2 yr^{-1}, max: 2.6 mg cm^2 yr^{-1}). Diatom valve density for the most part mirrored the productivity increases shown by BSi flux, however UC1 was more variable in the early 20th century (Figure 2.7).

The diatom communities of Upper and Lower Red Lakes cluster together in ordination space by lake based on each lake's distinct community composition (Figure 2.8). Sediment core trajectories over time are centered around the oldest sample except at the tops of the cores. Upper Red Lake has a greater abundance of benthic diatoms and the historical communities showed a significant shift decreasing in *Staurosira construens* Ehrenberg and *S. venter* (Ehrenberg) Cleve & J.D.Möller, replaced by a modern community increasing in *Achnanthidium minutissimum* (Kütz.) Czarn., *and Navicula cryptotenella* Lange-Bertalot over the late 20th century. Diatom communities of Lower Red Lake contrast with those of Upper Red Lake and have about 15% greater abundance of planktonic species such as *Asterionella formosa* Hassall*, Aulacoseira ambigua* (Gunow) Simonsen*, A. granulata* (Ehrenberg) Simonsen, *Lindavia bodanica* (Eulenst. ex Grunow) Nakov et al.*,* and *Stephanodiscus niagarae* Ehrenberg (Figures 2.7-2.13).

Diatom communities in both Upper Red Lake cores showed a historical shift in the late 1960s (Figures 2.9 and 2.10). Changes in the dominant diatoms for these cores include increased *Achnanthidium minutissimum* (Kützing) Czarnecki, *Aulacoseira ambigua*, *Navicula cryptotenella* Lange-Bertalot, and

other benthic species that replaced the earlier dominant *Staurosira construens* Ehrenberg and to a lesser degree *S. venter* (Ehrenberg) Cleve & J.D.Möller and *Staurosirella pinnata* (Ehrenberg) D.M.Williams & Round. Constrained cluster analysis of the Lower Red Lake cores did not identify any significant shifts in diatom communities when compared against a broken stick model (Figures 2.11-2.13).

The range of historical diatom-inferred TP (DI-TP) across the Red Lakes over the last 200 years was 30.1 *μ*g/L to 52.0 *μ*g/L; the average DI-TP for UC1 and UC4 was 38.6 *μ*g/L while the average for LC1, LC2, and LC3B was 43.7 *μ*g/L (Figure 2.7). The proportion of variation in the diatom data that can be explained by TP (λr/ λp) for LC1, LC2, LC3B, UC1, and UC4 was 0.24, 0.41, 0.26, 0.20, 0.47 respectively. These are low values, telling us that TP was not a strong driver of change in the diatom community. While ANOVA revealed a significant difference between the Upper and Lower Red Lake modern DI-TP, historical DI-TP, and monitored TP, Tukey pairwise comparisons revealed the only significant difference was between basins for the monitored TP values (Figure 2.14, Tables 2.2, 2.3).

2.4 Discussion

Lake management around the planet is compounded by anthropogenic stressors; point-source and non-point source nutrient enrichment, invasive plants and animals, changing water levels, climate change, and atmospheric deposition. These impacts result in ecological degradation from loss of sensitive species, increased harmful algal blooms, and disrupted ecosystem function and services.

When lakes are degraded and exceed monitoring criteria, lake managers use increased monitoring, modeling, and Total Maximum Daily Load plans to address nutrient loading and restore water quality. If lake conditions can be attributed to naturally high-water quality measurements that exceed standards for lakes and rivers of Minnesota, a variance or site-specific standard can be proposed based upon scientific and historical evidence (Minn. R. 7050.0220 subp. 7, MPCA 2011, Bouchard et al. 2017).

Over the last 20 years, Upper and Lower Red Lakes have exceeded total phosphorus and chlorophyll-a impairment criteria for the Northern Lakes and Forests ecoregion (30 μg L^{-1} TP, 9 μg L^{-1} chl-a, 2 m Secchi depth, Heiskary and Wilson 2008, Anderson 2017). In addition to having impaired waters, cyanobacteria blooms have been frequently reported, especially in Upper Red Lake. The watershed of Upper and Lower Red Lake has had disturbances, albeit minimal, including post-European settlement including timber harvest (Albrecht and Thomas 1977), peatland drainage (Volstead Act of 1906), and damming of the lakes' outflow. While 1/3 of the tributaries to the Red Lakes were also identified as impaired, ecological investigation revealed that these impairments were due to natural causes (Dollinger et al. 2017). Establishing the longer-term ecological trajectory of Upper and Lower Red Lake better informs lake managers as to whether the Red Lakes would benefit from increased monitoring or remediation efforts (Suding and Gross 2006).

Paleolimnology has been used to reconstruct ecological changes in lakes for a wide variety of applications including changes to the algae community and

nutrient impairment (Edlund et al. 2009). Furthermore, paleolimnology was a crucial tool in the Minnesota “weight of evidence” approach to establish lake nutrient criteria in three ecoregions of the state (Ramstack et al. 2003, Ramstack et al. 2004, Heiskary and Wilson 2008). For Lake Winona, Minnesota, paleolimnology was critical in determining historical lake phosphorus levels and an ecological trajectory of nutrient enrichment, which led to hyper-eutrophic conditions (Edlund and Ramstack 2009). Paleolimnology showed that modern conditions are extremely altered compared to the historical conditions and informed lake managers to take action through an adaptive management approach including a TMDL, invasive species removal, and phosphorus precipitation (MPCA 2011). Here we used a multiple sediment core approach to derive the paleo-ecology of Upper and Lower Red Lake in order to determine any ecological trajectories or changes within the lakes.

Paleolimnological evidence for the past 200 years showed only minor biogeochemical changes in Upper and Lower Red Lakes. Dry mass accumulation rates increased slightly over time in most cores, and sediment phosphorus, in particular the percentages of Fe-P and loosely bound P also showed gradual increases over the 20th century). Historical events may have contributed to the increase in long-term sediment P and sedimentation rates during the early logging, damming of the lake, and draining of the peatlands. However, the MPCA currently estimates that only 32% of phosphorus input to the lake is from watershed tributaries while 47% is from atmospheric deposition (RMB 2017). With most watershed changes occurred in the late 19th and early

20th century, their effects, if any, should have subsided with reforestation. Using BATHTUB lake models, RMB (2017) suspects that low level P enrichment and sediment deposition to the lake is aeolian in origin. The increase in sediment Fe-P could be indicative of increased summertime anoxia events driven by warmer temperatures and decreased regional wind speeds (Reavie et al. 2017). Another indicator of a gradual increase in productivity is the carbon (%) and nitrogen (%) isotopes increasing above historical conditions over the 20th century (Leavitt and Hodgson 2001). Furthermore, the slight decrease in C:N could indicate increased algal productivity or sediment diagenesis. Overall, phosphorus and sediment accumulation and lake productivity appear to be increasing slightly in the lakes over the 20th century.

A notable change in the diatom community from Upper Red Lake occurred in the late 1960s, with increases in *Achnanthidium minutissimum* and *Navicula cryptotenella* (Figures 11 and 12). These diatoms differ from the other dominant diatoms by virtue of their benthic life strategies, *N. cryptotenella* often living in the benthos and *A. minutissimum* often epiphytic on other algae or macrophytes (Wehr et al. 2014). While an increased signal of benthic diatoms could be indicative of frustule dispersion during sediment mixing, the increase in *A. minutissimum* may also coincide with increases in filamentous algae or aquatic macrophytes.

These minor paleo-ecological changes in Upper and Lower Red Lake, however, contrast other biogeochemical evidence indicating ecological stability for the previous 200 years. The diatom record of Lower Red Lake showed no

significant community shifts, and there were no significant differences or trends in the diatom-inferred total phosphorus or biogenic silica for either lake. The average modern and historical diatom-inferred total phosphorus for each basin was between the monitored averages for each basin (Anderson 2017), with no differences between the DI-TP and monitored TP values. This suggests the nutrient perspective represented by diatom ecology is functionally similar between the two basins. The fossil pigment data similarly show no major community shifts within cyanobacteria and the greater algal community. Beneficial factors that might explain why the modern ecology of Upper and Lower Red Lakes is similar to the historical ecology include nearly a century since any major watershed disturbances, the Red Lakes watersheds have overall low portions of land in development, and a relatively small watershed to lake surface area ratio.

The paleo and modern water chemistry for Upper Red Lake and Lower Red Lake were consistent within each respective basin. One important characteristic which differentiates the two basins is mean water depth. Upper Red Lake is half the mean depth of Lower Red Lake, 3.6 m vs. 7 m, respectively (Anderson 2017), which contributes to greater wind-driven mixing in Upper Red Lake. The effects of this are pronounced in the higher inorganic content of sediments and large portions of the basin with non-conformable sediment profiles. Diatom ecology also separates the two lakes with higher proportions diatoms characteristic of dimictic lakes in Lower Red Lake, whereas Upper Red Lake had higher proportions of diatoms characteristic of shallow polymictic lakes. Relative

to Lake of the Woods, these differences are subtle compared to the basin differences observed in Lake of the Woods (Rühland et al. 2010, Reavie et al. 2017). Mineral bound phosphorus and inorganic sediments were also higher in Upper Red Lake compared to Lower Red Lake indicating that lake depth may a primary determining factor for the spatial distribution of sedimentation within the two basins. Within Upper Red Lake, the eastern most sediment cores were non-conformable suggesting frequent or intensive sediment resuspension. More frequent mixing and lower overall P burial likely affect monitored total phosphorus, chlorophyll-a, and Secchi depth, which also indicated greater productivity in Upper Red Lake. Overall, the sediment evidence suggests that wind-driven mixing plays a larger role in the ecology of Upper Red Lake compared to Lower Red Lake.

Understanding depth, wind, and sediment dynamics is not only important for informing the ecological management of a lake, but also necessary for site selection in paleolimnological studies. Bachmann et al. (2018), showed inconsistences between models and paleo-measurements of sediment accumulation and P concentrations. Lakes near each other may reflect environmental change differently (Forbes and Hickman 1981); furthermore, separated basins within the same lake can yield different sedimentary records and varying water quality reconstructions (Rühland et al. 2010, Reavie et al. 2017). Given the complex nature of working in productive shallow lakes (Heathcote et al. 2015), the evidence presented here and in other studies

highlights the importance of a multi-core, multi-basin approach to establish reliable historical reconstructions.

While Upper and Lower Red Lakes may have some geochemical sedimentation differences, they both recorded uniform but subtle increased organic sediments, increased phosphorus accumulation, increased diatom productivity, and increased algal productivity beginning in the early 20th century continuing to the present day. Mirroring a decline in mineral bound phosphorus, the proportion of phosphorus bound by iron increases over this time; iron-bound phosphorus is one of the more mobile sediment P fractions and often is greatest near the sediment water interface. This shift begins around the time of the greatest land use changes within the watershed; logging, draining of wetlands, and damming of the lake. We know these watershed activities can be associated with phosphorus contributions to aquatic environments (Nieminen 2004), and that shallow lakes in particular take longer to recover (Jeppesen et al. 2005, Jeppesen et al. 2007), however, since the early 20th century, these activities have been largely curtailed and the lakes continue to increase in available nutrients and productivity. As Edlund et al. (2017) suggested for nearby Lake of the Woods, regional climate forcing may be driving changes in the Upper and Lower Red Lakes. Alluded to earlier, the decreasing trend in regional wind (Reavie et al. 2017) could increase the number of anoxic-stratification events contributing to internal loading (Nürnberg 1995). While wind-driven sediment resuspension likely occurs more frequently in Upper Red Lake, and likely contributes significantly to internal phosphorus loading (James 2017b), greater

quantities of phosphorus can be released through anoxic stratification on a single event basis (James 2017a). More work is needed to determine if stratification events are increasing with climate change, and dynamic phosphorus models are needed to better understand the interplay between decreased wind mixing and increased anoxia. Increased winter air temperatures can lead to longer open water seasons and longer algae growing seasons (O'Beirne et al. 2017).

In addition to potential climate-driven increased internal loading, the Red Lakes may be subject atmospheric deposition of dust (Zhu et al. 2019). Dust deposition has shown to contribute particulate matter to lakes over large distances (Mladenov et al. 2009) and a pilot study has shown aeolian dust deposition in Minnesota to be a carrier of phosphorus (Engstrom et al. 2019). More work is needed understand how the upwind agricultural areas may be contributing phosphorus and mineral matter to the Red Lakes watershed.

Given this paleolimnological evidence and 20 years of modern water quality monitoring efforts that show no historical trends, we conclude that Upper and Lower Red Lakes have likely always exceeded the current regional total phosphorus standard (30 µg L^{-1}, Minnesota Administrative Rules:7050.0222). With this understanding of Upper and Lower Red Lakes history, we suggest the establishment of a site-specific standard for the Red Lakes (Minnesota Administrative Rules:7050.0222). For Upper and Lower Red Lakes we propose adopting the 75th percentile based on the 20 years of growing season (May-October) monitored water quality data as site specific standards for total phosphorus, chlorophyll-a, and phaeophytin corrected chlorophyll-a (Table 2).

We propose using the 25th percentile of the monitored Secchi depth (Table 2). As such, standards for Lower Red Lake should be set at 45 *µ*g/L TP, 14 *µ*g/L chlorophyll-a, 16 *µ*g/L pheophytin corrected chlorophyll-a, and 0.91 m Secchi depth, and standards for Upper Red Lake should be set at 54 *µ*g/L TP, 20 *µ*g/L chlorophyll-a, 17 *µ*g/L phaeophytin corrected chlorophyll-a, and 0.61 m Secchi depth. An alternative solution would be to regulate Upper and Lower Red Lakes as part of the North Central Hardwood Forests ecoregion (60 *µ*g/L TP, 20 *µ*g/L chlorophyll-a, 1 m Secchi depth). Our recommendations follow methods recommended for developing site-specific in Minnesota using a combination of monitored data and paleolimnological inference (MPCA 2011, Heiskary and Wasley 2011, Bourchard et al. 2017)

2.5 Conclusion

The geochemical and biotic sediment record of Upper and Lower Red Lakes does not indicate any major changes to the lakes' ecology over the last 200 years. The sediment record records minor effects from watershed activity in the early 20th century to the Red Lakes and slow but minor increases in overall productivity that may be linked to climate forcing and activities outside the watershed. Combined with 20 years of monitoring data that also show no significant trends in water quality parameters, the productive nature of the lakes, and strong potential for in-lake sediment resuspension, alternative nutrient standards should be considered for the future regulation of Upper and Lower Red Lake.

Chapter 3

Paleolimnology and Resurrection Ecology: The Future of Reconstructing the Past

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Abstract

Paleolimnologists have utilized lake sediment records to understand historical lake and landscape development, timing and magnitude of environmental change at lake, watershed, regional and global scales, and as historical datasets to target watershed and lake management. Resurrection ecologists have long recognized lake sediments as sources of viable propagules ("seed or egg banks") with which to explore questions of community ecology, ecological response, and evolutionary ecology. Most researchers consider *Daphnia* as the model organism in these efforts, but many other aquatic biota, from viruses to macrophytes, similarly produce viable propagules that are incorporated in the sediment record but have been underutilized in resurrection ecology. The common goals shared by these two disciplines have led to mutualistic and synergistic collaborations - a development that must be encouraged to expand. We give an overview of the achievements of paleolimnology and the reconstruction of environmental history of lakes, review the untapped diversity of aquatic organisms that produce dormant propagules, compare *Daphnia* as a model of resurrection ecology with other organisms amenable to resurrection

studies, especially diatoms, and consider new research directions that represent the nexus of these two fields.

3.1 Lake Sediments as Environmental and Evolutionary Archives

Lakes are a dominant feature on much of the earth's surface with over 117 million lakes greater than 0.2 ha estimated to cover about 3.7% of the land area (Verpoorter, Kuster, Seekell & Tranvik, 2014). Importantly almost every lake accumulates sediments at rates of millimeters to 10s of cm per year in conformable patterns of deposition. Paleolimnologists rely on the ability to sample, date, and analyze physical and biogeochemical signals preserved in the sediments to determine lake and landscape evolution, environmental change in lakes, and lake response to local, regional and global drivers. In contrast to terrestrial sediments that are often disturbed by erosion and bioturbation, aquatic sediments accumulate at measurable rates because they are often buffered from physical, chemical, and biotic disturbance. Because of these features, many different proxies of the sediment record are preserved that can be analyzed with a higher temporal resolution when compared to terrestrial paleontology.

Ingrained in the life history of many organisms adapted to living in lakes are dormancy strategies that result in resistant propagules specialized to survive or perennate at the sediment-water interface. Unsuccessful termination of the dormant phase or entrainment back to the water column can result in propagules being permanently buried in the sediment column. This "egg bank" (Cáceres & Hairston, 1998) has been a rich repository of information for resurrection ecologists.

Resurrection ecology is a fast-moving field combining evolutionary biology, ecology, and paleobiology to study how terrestrial (Seddon, Moehrenschlager, & Ewen, 2014) and aquatic species (Brendonk & Meester, 2003) persist, propagate and evolve under the forces of ecological change. For some, resurrection ecology may suggest de-extinction by implanting fragments of ancient DNA isolated from fossil tissues into the oocytes of modern organisms; however, this process has not been viably successful (Folch, Cocero, Chensé et al., 2009) and is not the focus of this paper. Although many terrestrial organisms produce dormant structures (e.g., seeds), here we focus on freshwater aquatic organisms whose viable dormant propagules coupled with the rich paleo-limnological sediment record and its high temporal resolution (see above) provide opportunities to study populations, organisms, and environments of the past. Many zooplankton and microbes have evolved unique dormant life stages that survive or persist in lake sediments (Hairston & Fox, 2009). Organisms deposit dormant stages in lake sediments to survive unfavorable growing conditions such as desiccation in ephemeral ponds and overwintering in larger water bodies. After the unfavorable growing conditions pass, bioturbation and wind driven turbulence are mechanisms that mix sediments into the water column providing triggers to end dormancy. Cáceres and Hairston (1998) describe the dormant stages at the sediment-water interface that can mix back into the water column as the active egg bank, whereas those permanently trapped in the lake sediments form the inactive egg bank. It is the viable dormant stages of the inactive egg bank that provide a unique source of populations from the past.

Resurrection ecologists can bring back to life viable dormant propagules of ancient aquatic organisms that allow phenotypic or genomic characterization rather than piecing together fragmentary fossil DNA. Resurrected organisms provide exceptional opportunities to study evolutionary processes, are a potential source of extinct species or lineages, and can be used to test paleo-proxies of environmental change (reviewed in Orsini, Schwenk, De Meester et al., 2013).

The same conditions that allow egg banks to persist in lake sediments—dark, cold, anoxic—are also conducive to preservation of the many physical and biogeochemical signatures or proxies that paleolimnologists use to understand timing and magnitude of ecological change in lakes. Paleolimnology is a well-established field where a wide variety of abiotic (e.g., bulk density, dry mass, radioactive isotopes, mineralogy) and biotic proxies (e.g., fossils, species abundances, presence/absence, pigments) preserved in the sediments are analyzed to reconstruct ecosystem change at time scales ranging from interannual to decadal to millennial. Intact lacustrine sediment cores from 10s of centimeters to 100s of meters in length can be recovered from depositional basins using simple line-operated devices to drilling rigs (Wright, 1991). To develop date-depth relationships in cores, well-established dating techniques including radioisotopic (especially ^{14}C, ^{210}Pb, and ^{137}Cs) and stratigraphic (e.g., pollen) methods are used (Appleby, 2001). Careful collection and dating of sediment cores ensures that a conformable sedimentation record has been sampled, and allows the many physical and biogeochemical proxies to be confidently analyzed.

The next step in paleoecological investigations is the analysis of proxies to develop precise reconstructions of ecosystem change; this is the framework of paleolimnology and is fundamental for providing environmental context for resurrection ecology. Physical, geochemical, and biological proxies including organic remains such as diatom, cyanobacteria, and zooplankton subfossils, and their dormant propagules, can be analyzed in lake sediments. The information gleaned by proxy presence/absence, abundance, morphology, and condition hold certain relevance for the paleolimnologist, but the same remains and accompanying data will also provide detailed and crucial paleoenvironmental information needed to interpret depositional environment, ecological setting, and resource dynamics for the resurrection ecologist.

Single proxy analyses are still used in paleolimnology to provide specific stressor identification such as historical deposition of heavy metal mining waste in lakes (Kerfoot, Lauster, & Robbins, 1994). Since the 1980s, paleolimnologists have applied quantitative reconstruction techniques to estimate individual historical lake conditions of interest such as pH, salinity, nutrient levels using diatoms (Fritz, Juggins, Battarbee, & Engstrom, 1991; Dixit & Smol, 1994; Bennion, Juggins, & Anderson, 1996; Ramstack, Fritz, Engstrom, & Heiskary, 2003), and dissolved oxygen using chironomid remains (Brodersen & Quinlan, 2006). Quantitative inference models are typically developed by sampling water quality and surface sediments in many lakes (the training set) that captures a gradient of the environmental variable of interest. Exploratory multivariate techniques are used to identify which environmental factors independently and

significantly explain, for example, diatom abundance and distribution in the lakes. Predictive models are developed using techniques such as weighted averaging regression and calibration so a subfossil diatom assemblage can be used to predict, for example, historical salinity (Fritz et al., 1991) and total phosphorus levels (also referred to as diatom-inferred total phosphorus; Ramstack et al., 2003). Recent criticisms of quantitative inference modeling have resulted in appropriate precautions (Juggins, 2013), such as training set sample size and age appropriateness of the flora (Reavie & Edlund, 2013). Given that multiple factors driving assemblage change, not all of the taxa within a flora are sensitive to the constituent of interest, and different models are not easily transferable (Juggins, Anderson, Hobbs, & Heathcote, 2013).

A more powerful approach in paleoecology is multiproxy analysis of cores (Birks & Birks, 2006) to reconstruct past lake and watershed conditions. As its name implies, multiproxy analysis relies on simultaneous analysis of a multiple physical and biogeochemical proxies to develop a more complete understanding of timing and magnitude of ecological change, which might include atmospheric deposition, shifts in habitat structure, lake eutrophication, and resulting food web interactions (Sayer, Davidson, Jones, & Langdon, 2010). For example, in Australian billabongs, multiproxy analysis revealed how the progression of eutrophication impacted diatom, macrophyte, cladoceran and chironomid communities (Davidson, Reid, Sayer, & Chilcott, 2013).

Multiproxy studies can also be extended to include whole basin historical reconstructions. For example, diatoms, fossil algal pigments, phosphorus

fractions, and biogenic silica were analyzed in a series of 18 cores from a natural riverine lake bordering Minnesota and Wisconsin (USA). Multiproxy and whole basin techniques (Engstrom & Rose, 2013) were melded to develop a precise historical record of nutrient loading, nutrient availability, lake productivity (biogenic silica), and ecological change in primary producer communities, all linked to documented land-use changes (e.g. logging, agriculture, urbanization) in the watershed (Triplett, Engstrom, & Edlund, 2009; Edlund, Engstrom, Triplett et al., 2009a; Edlund, Triplett, Tomasek, & Bartilson, 2009b). In addition to their use as environmental archives of human disturbance, paleolimnologists have used lake sediments to understand the timing of exotic species introductions (Hairston, Perry, Bohonak, et al. 1999, Edlund, Taylor, Schelske, & Stoermer, 2000), speciation (Theriot, Fritz, Whitlock, & Conley, 2006), and post-glacial succession (Engstom, Fritz, Almendinger, & Juggins, 2000).

Single lake paleolimnological studies have also given way to multi-lake studies that consider among-lake variation in ecological response, regional trend assessment, and large-scale syntheses of stressor impacts. Climate-mediated shifts in diatom assemblages showed temporal variability and differences in magnitude of change in both regional (Shinneman, Umbanhowar, Edlund et al., 2016) and hemispheric scales (Rühland, Paterson, & Smol, 2008). Among-lake differences in stressor response become very evident in multi-lake studies. Carbon burial, a proxy for in-lake productivity and terrestrial C sources, was analyzed in over 100 lakes and showed that ecoregional patterns of landuse and development and subsequent nutrient enrichment overwhelmed other potential

drivers of carbon sequestration such as climate change (Anderson, Dietz, & Engstrom, 2013; Dietz, Engstrom, & Anderson, 2015). Contrasting patterns of C burial and phosphorus accumulation in two Minnesota lakes were correlated with the lakes' contrasting development, land use, and lake eutrophic histories. Importantly, these contrasting lake histories provided the ecological framework for interpreting temporal changes in population genetic structure of *Daphnia* based on paleogenetic analysis of dormant eggs (Frisch, Morton, Culver et al., 2017).

A recent collaboration among paleoecologists posed 50 priority questions to guide the future of paleolimnology (Seddon, Mackay, Baker et al., 2014). Key research directions that were identified included Anthropocene human-environment interactions, biodiversity and conservation, biodiversity changes over multiple time scales, use of multiple lines of evidence (multi-proxy and across spatial and temporal scales), and new developments in paleoecology. Among the questions raised were many that are best answered using resurrection ecology techniques, but more relevant to this paper were the many questions and developments that needed combined efforts from paleolimnologists and resurrection ecologists.

In this review, we explore this key link that is developing between paleolimnology and resurrection ecology. We first discuss the use (to date) of the primary model organism, *Daphnia*, in resurrection ecology, noting both the beneficial and limiting characteristics of this model organism. We then briefly discuss other organisms that produce dormant stages that are deposited into

freshwater lacustrine sediments including their relevant life history strategies, viability of propagules, and the main utility of each group in paleoecological research. Other organisms that possess dormant stages, such as diatoms, have hardly been utilized in resurrection ecology despite a proven record of utility in paleolimnology as indicators of change in habitat, pH, salinity, and nutrient level. These environmental changes are driven by large-scale regional and global stressors that are among the strongest selective pressures affecting lakes and their biota (Frisch et al., 2017). We conclude by exploring areas of research that represent the nexus of resurrection ecology and paleolimnology including responses of single species to environmental drivers, assessment of community-level and multiple organism responses to change, development of better mechanistic understanding of environmental and evolutionary change, and new strategies and technologies available to address these areas.

3.2 *Daphnia* A Model Organisms in Resurrection Ecology

Since the term resurrection ecology was coined (Kerfoot, Robbins, & Weider, 1999; Kerfoot & Weider, 2004), the planktonic crustacean *Daphnia* (water flea) has been developed as a model organism in resurrection ecology. Early studies pioneered experimental research on resurrected *Daphnia* (*e.g.*, Hairston et al., 1999; Cousyn, De Meester, Colbourne et al., 2001) to understand evolutionary adaptation to increasing anthropogenic impacts on the environment. In contrast to comparing phenotypic and genomic responses of traits from spatial populations that differ in their environments as well as their genetic background, evolutionary adaptation to environmental change can be directly observed in

resurrected temporal populations. Experimental evolution, an alternative to resurrection ecology where resurrected isolates are unavailable, is typically applied to unicellular organisms such as bacteria or yeast or multicellular organisms with short generation times such as *Drosophila* (reviewed in Bell, 2016). However, more recently, experimental evolution using *Daphnia* has gained momentum, with studies of asexually propagated mutation accumulation lines generated over a maximum number of 100 generations (Xu, Schaack, Seyfert et al., 2012). In contrast, resurrection ecology is geared towards the study of natural *Daphnia* populations shaped by the complexity of the biotic and abiotic environment over timeframes that could span decades or even centuries (Frisch et al., 2014) and thus may represent thousands of asexual generations.

Dormant eggs of *Daphnia* generally result from sexual reproduction except for obligately asexual lineages that occur at higher latitudes (reviewed in Dufresne, Marková, Vergilino et al., 2011). In *Daphnia*, two eggs are encapsulated together in an ephippium, that form egg banks in the sediment of lakes and ponds. Densities of *Daphnia* ephippia deposited in egg banks can reach > 10,000 ephippia m^{-2} (Carvalho & Wolf, 1989; Cáceres, 1998). Dormant eggs of *Daphnia* can be hatched for culturing in the laboratory. However, egg viability is impacted by age and environmental conditions of the sediment (Weider, Lampert, Wessels et al., 1997), limiting the number of hatchlings that can be obtained from several centuries old eggs (Morton, Frisch, Jeyasingh, & Weider, 2015). Owing to *Daphnia's* cyclical parthenogenetic life cycle (Decaestecker, De Meester, & Mergeay, 2009), clonal cultures of genetically

identical individuals (clonal lineages) can be established in conditions that suppress induction of sexual reproduction and male formation.

To hatch historic eggs from distinct time periods, ephippia are removed from dated sediment, decapsulated and exposed to conditions that induce development. Hatching success is constrained by egg viability and drops with age of the eggs, with >75% hatching from sediment as old as 20 years (Weider et al., 1997) to greatly reduced (<<1%) hatching events in centuries old layers (Frisch et al., 2014). Because of this constraint, existing studies are typically focussed on resurrected *Daphnia* < 70 years old (*e.g.,* Hairston et al., 1999; Cousyn et al., 2001; Jansen, Geerts, Rago et al., 2017), with some exceptions (120 years (Cáceres, 1998), 600-700 years (Frisch et al., 2014)).

Life history experiments of resurrected clonal lineages support the capacity for adaptive responses of *Daphnia* to the environment, for example to toxic algae (Hairston et al., 1999), host-parasite interactions (Decaestecker et al., 2009), eutrophication (Frisch et al., 2014), temperature change (Gabriel & Lampert, 1985; Geerts, Vanoverbeke, Vanschoenwinkel et al., 2015), fish predation (Cousyn et al., 2001), multiple environmental stressors (Orsini, Spanier, & De Meester, 2012), or have provided insight into the evolution of phenotypic plasticity (Henning-Lucas, Cordellier, Streit, & Schwenk, 2016). The development of high throughput DNA sequencing allows phenotypes of resurrected *Daphnia* to be associated with single nucleotide polymorphisms (SNPs) of whole genomes using Whole Genome Association studies (GWAS, Miner, De Meester, Pfrender et al., 2012; Orsini et al., 2012), or with

transcriptomic patterns of the same resurrected clones (Roy Chowdhury, Frisch, Becker et al., 2015; Lack, Weider, & Jeyasingh, 2018). To identify putative quantitative trait loci (QTLs), resurrected clones with contrasting phenotypes are crossed that represent different time points of the same populations. Such an approach is currently being explored and has recently produced F2 mapping panels for quantitative genetic analyses (LJ Weider & PD Jeyasingh, personal communication).

As detailed above, *Daphnia* species have proven an excellent organismal group to provide the foundation for and expand the field of resurrection ecology. The ability of their dormant eggs to survive centuries, a cyclical parthenogenetic life cycle with short generation times for genetically identical replicates, environmental sensitivity, established genetic and genomic resources, and their easy culturing have made *Daphnia* a model organism leading the field of resurrection ecology. However, use of a single organismal group limits the examination of trophic interactions (Carpenter, Kitchell, & Hodgson, 1985), co-evolutionary dynamics (Kinnison, Hairston, & Hendry, 2015), and *Daphnia* or other macroinvertebrate communities might not be sensitive to perturbations as other communities such as diatoms (Justus, Burge, Cobb, Marsico, & Cobb, 2016).

3.3 Other Freshwater Organisms With Dormancy Stages

Ranging from macroinvertebrates, algae, and phages, viable long-term resting stages have been observed for a wide variety of freshwater organisms. Here we evaluate the applicability of several freshwater organisms that have also

been used as paleolimnological indicators. Based on microbial ecology, Lennon and Jones (2011) characterized propagule dormancy into three stages: initiation, dormancy, and resuscitation. Under this dormancy framework, initiation of asexually produced microbial spores could be triggered by resource limitation or spontaneously; in higher organisms, propagules result from sexual or asexual reproduction in response to biotic cues including predation (Gyllström & Hansson, 2004). Dormancy is associated with physiological changes, energetic costs, and ecological trade-offs (Lennon & Jones, 2011; Alekseev, De Stasio, & Gilbert, 2007; Shoemaker & Lennon, this issue), and its duration can range from weeks to decades or even centuries (Alekseev et al., 2007). Resuscitation of dormant cells, like initiation, is triggered by environmental cues (Sicko-Goad, Stoermer, & Kociolek, 1989; Gyllström & Hansson, 2004) or in the case of microbes, dormancy release can also be spontaneous (Lennon & Jones, 2011). We review the dormancy strategies and paleoecological significance of freshwater organisms that are commonly used in paleolimnology and that include dormant propagules as part of their ecology and life history (Table 3.1). For greater detail on resting stage cytology, metabolism, and dispersal see Ellegaard and Ribeiro (2017) for phytoplankton, and Gyllström and Hasson (2004) and Alekseev et al. (2007) for aquatic invertebrates.

Many species of freshwater zooplankton undergo dormancy as a life history strategy and produce resistant propagules for community resilience and dispersal which enables them to survive digestion or transport by birds (Fryer, 1996; Frisch, Green, & Figuerola, 2007). Gyllström and Hansson (2004) provide

an extensive review on Cladocera, Copepoda, and Rotifera dormancy, organisms that are also commonly used in paleolimnology (Smol, Birks, & Last, 2001). A discussion on *Artemia* as a suitable organism for resurrection ecology is discussed in this issue (Lenormand, Nougué, Jabbour-Zahab, et al., 2018). The dormant eggs of calanoid copepods have provided a resource for resurrection ecology that several studies have taken advantage of (e.g. Hairston et al. 1995; Derry, Arnott, & Boag, 2010; Jiang, Zhao, Xu et al., 2012). Zooplankton generally produce dormant propagules sexually, as fertilized diapausing eggs; however, Cladocera and Rotifera can also produce dormant propagules parthenogenetically. Dormant eggs of the Anomopoda, a suborder of Cladocera which includes *Daphnia,* are enveloped in a protective chitinous structure known as an ephippium (Figure 3.1a). Gyllström and Hansson (2004) summarized a variety of abiotic and biotic trigger mechanisms for inducing dormant egg production in zooplankton including resource limitation, seasonality, crowding, and predation. Zooplankton propagules have an increasingly well-documented record of longevity in lake sediments. Frisch, Morton, Roy Chowdhury et al. (2014) hatched *Daphnia* eggs that were dormant in lake sediments for up to ~600-700 years. Hairston, Van Brunt, Kearns, and Engstrom (1995) found dormant calanoid copepod dormant eggs to be viable for over 300 years. Rotifera appear to have a shorter dormant egg longevity of 35 to 40 years (Nipkow, 1961; Marcus, Lutz, Burnett, & Cable, 1994). In all studies reviewed (n=49), Gyllstöm and Hansson (2004) found that environmental cues (temperature, light, dissolved oxygen) most often associated with seasonality could trigger resuscitation of

cladoceran, copepod, and rotifer propagules. Hatching rates of dormant zooplankton propagules are negatively correlated with sediment age and significantly inhibited by environmental stressors such as metal contamination (Rogalski, 2015).

Apart from dormant propagules, most zooplankton also produce physical remains that are incorporated into the sediment record and are used by paleoecologists. Cladocera have a chitinous exoskeleton (Figures 3.1;b, c), which allows body parts such as carapaces, claws, and spines to persist in lake sediments. Cladoceran remains from lake sediments have been used to track changes in climate (Lotter, Birks, Hoffman, & Marchetto, 1997; Smol, Wolfe, Birks et al., 2005), lake level (Hyvärinen & Alhonen, 1994), trophic state (Boucherle & Züllig, 1983; Bos & Cumming, 2003), lake acidity (Nilssen & Sandoy, 1990), and non-native species invasion (Keilty, 1988). Copepod exoskeletons remain in the lake sediment records, but do not preserve as well compared with Cladocera (Rautio, Sorvari, & Korhola, 2000), therefore copepod diapausing eggs (Bennike, 1998) and spermatophores have been suggested as a useful paleolimnological indicators of lake level (Borromei, Coronato, Franzén, et al. 2010). Copepod spermatophores have also been used in conjunction with zooplankton community data for reconstructing anthropogenic eutrophication (Findlay, Kling, Rönicke, & Findlay, 1998). Rotifers are primarily identified from lake sediments by their loricas. Rotifer loricas have been used to reconstruct early post-glacial community succession (Swadling, Dartnall, Gibson et al., 2001) and nutrient dynamics of lakes (Findlay et al. 1998; Turton & McAndrews, 2006).

In addition to Cladocera, other Branchiopoda that form diapausing eggs include Anostraca, Conchostraca, Mysida, Notostraca, and Spinicaudata (Fryer, 1996). Anostracan remains have been used to reconstruct lake salinity (Bos, Cumming, & Smol, 1999); however, these groups are not commonly used in paleoecological reconstructions or resurrection ecology. Ostracods are a group of freshwater microcrustaceans commonly used in paleolimnology and that form diapausing eggs. Hairston, Dillon, and De Stasio, Jr. (1990) found that diapause initiation in ostracods occurred during the winter in response to temperature and photoperiod reduction. Ostracod diapause eggs are resistant to freezing (Theisen, 1966) and desiccation (McLay, 1978). McLay (1978) found that when favorable growth conditions returned, some species would develop immediately whereas other species underwent a prolonged dormancy period. In paleolimnology, ostracod abundance determined from light microscopic identification of remnant shells (Figure 3.1d) has been used to reconstruct fluctuations in lake level (Viehberg, 2004) and gradients of ionic concentrations in modern limnology (Van der Meeren, Almendinger, Ito, & Martens, 2010). Ostracod shell chemistry is an especially powerful tool in paleolimnology. Isotope composition of ostracod shells directly reflects water chemistry at the time of shell formation so shell chemistry has been used to reconstruct paleosalinity, precipitation, and temperature (Xia, Haskell, Engstrom, & Ito, 1997). While ostracod eggs have not been reported from deep water sediment cores, they have been collected and hatched from ponds (Rossi, Albini, Benassi, & Menozzi, 2012). Members of the Porifera, the freshwater sponges, produce dormant

stages known as gemmules (Rasmont, 1954). Gemmule formation is parthenogenetic and can be triggered by increasing osmotic pressure; increasing temperature can induce gemmule hatching (Simpson & Fell, 1974). Light, season, and nutrient availability influence the size and thickness of gemmules, which determines their resilience. Porifera gemmules have been found to be viable from 25-year-old lake sediments (Harrison, 1974). Freshwater sponges deposit two types of siliceous spicules; gemmoscleres are smaller and used for species identification, whereas megascleres are enumerated for population estimates (Harrison, 1988). Freshwater sponges have been used as indicators of alkalinity (Harrison & Harrison, 1979) and paleosalinity (Cumming, Wilson, & Smol, 1993), and the density and thickness of spicules have been related to silica dynamics (Jewel, 1939; Kratz, Frost, Elias, & Cook, 1991).

A wide variety of unicellular organisms are known to form dormant stages in lake sediments including phages, bacteria, cyanobacteria, dinoflagellates, and diatoms. Lennon and Jones (2011) provide a comprehensive review of the diversity of initiation, dormancy, and resuscitation found in heterotrophic bacteria; however, they did not examine cyanobacteria in detail. Cyanobacteria are photosynthetic bacteria and many taxa vegetatively form dormant propagules called akinetes (Figures 3.1; f, g), which are fortified cells that can survive desiccation and adverse growing conditions (Miller & Lang, 1968; Yamamoto, 1975; Livingstone & Jaworski, 1980). Li, Watanabe, and Watanabe (1997) found that decreasing temperature was the primary factor inducing akinete formation. In laboratory experiments cyanobacteria failed to produce akinetes in the dark, and

akinetes become significantly less viable when exposed to temperatures below 20^{o}C (Agrawal & Singh, 2000). Akinetes have been resuscitated from 64-year-old lake sediments (Livingstone & Jaworski, 1980). Along with resuspension of lake sediments, environmental cues for the resuscitation of akinetes include increasing temperature and light (Rengefors, Gustafsson, & Ståhl-Delbanco, 2004). Cyanobacteria produce microfossils and pigment signatures in sediments that are used for paleoecological inference (Kling, 1998; Leavitt & Hodgson, 2002). Akinetes from lake sediments are used as a proxy for cyanobacteria abundance; Kling (1998) interpreted increasing cyanophytes as a paleo-proxy for increasing temperature and phosphorus. Taranu, Gregory-Eaves, Leavitt et al. (2015) used pigment concentrations obtained from 108 sediment cores to demonstrate increasing abundances of cyanobacteria as a response to eutrophication and climate change.

Preserved alongside cyanobacterial remains in sediments are viable cyanophages, viruses that infect and lyse cyanobacteria (Hargreaves, Anderson, & Clokie, 2013). Although virus-like particles are not used in paleoecological analyses, their utility in resurrection ecology to study predator-prey evolutionary relationships has been explored. Viable cyanophages were isolated from sediments up to 50 years old and used to infect cultures of the cyanobacterium *Microcystis* (Hargreaves et al., 2013).

Dinoflagellates are unicellular motile algae most commonly associated with red tides in coastal marine water, but they are also common freshwater phytoplankton. Some species are multi-trophic and able to switch between

autotrophy, herbivory, and parasitism. Many species are surrounded by an armored theca of cellulose plates (Carty, 2003). The dormant propagule produced by dinoflagellates is a cyst resulting from sexual reproduction (Dale, 1983). Dinoflagellates appear to undergo encystment after blooming as part of the annual life cycle (Heiskanen, 1993). The dinoflagellate cyst must undergo a period of maturation before hatching, which can be up to 5 months long (Binder & Anderson, 1986). Lundholm et al. (2011) resuscitated dinoflagellate cysts in lake sediments that were dated 90 years old. Germination of dinoflagellates appears to be induced by light exposure and suppressed by anaerobic conditions (Anderson, Taylor, & Armbrust, 1987). In paleolimnology studies, dinoflagellate resting cysts are enumerated in sediments using light microscopy (Livingstone 1984). Using cyst abundances, the community composition of dinoflagellates reflected land-use changes by indigenous people and European settlers in Ontario (McCarthy, Mertens, Ellegaard et al., 2011; McCarthy & Krueger, 2013).

Diatoms are photosynthetic microalgae and are the most diverse group of algae (Round, Crawford, & Mann, 1990). Among the microalgae, they are characterized by their ornamented two-part cell wall composed of biogenic silica (Figures 2.1;k, l). Diatoms produce two types of dormant vegetative stages including “resting spores” and “resting cells” (McQuoid & Hobson, 1995; Kaczmarska, Poulícková, Sato et al., 2013). Dormant spores are morphologically distinct and more heavily silicified than vegetative cells and are typically formed in response to decreasing nitrate (Davis, Hollbaugh, Seibert et al., 1980; Kuwata & Takahashi, 1990) and phosphate concentrations (Jewson, Granin, Zhdanov et

al., 2008). Diatom spore production is particularly common in coastal marine diatoms, although a few freshwater genera also form spores (Edlund & Stoemer, 1993; Edlund et al., 1996). Dormant cells (Figures 2.1; h, i, m) are formed in response to several environmental triggers including lower temperature and light conditions (Lund, 1954) and silica limitation following lake stratification. Silica limitation and stratification triggers diatoms to increase their sinking rate and shift their physiology to increased storage of carbohydrates and lipids and condensation of cell organelles around the nucleus (Gibson & Foy, 1988; Sicko-Goad et al., 1989; Gibson & Fitzsimons, 1990). It has also been suggested that some cold-favoring diatoms initiate dormant cells in response to warming temperatures (Nipkow, 1950). While dormant spores have shorter viability, on the order of weeks (Hargraves & French, 1975) to a year (Garrison, 1979), diatom dormant cells are viable for many decades while persisting in lake sediments (Stockner & Lund, 1970; Sicko-Goad, Stoermer, & Fahnenstiel, 1986). Dormant spores excyst under experimental nutrient replenishment (Jewson et al., 2008). Diatom dormant cell resuscitation appears to be less affected by nutrient levels (Hollibaugh, Seibert, & Thomas, 1981), rather greater recruitment occurs under increased light and temperature regimes (Figure 3.1j; Lund, 1954; Sicko-Goad et al., 1986; McQuoid & Hobson, 1995). The deposition and preservation of diatoms have enabled paleolimnologists to reconstruct historical changes in lake acidity (Battarbee, 1991; Camburn & Charles, 2000), salinity (Fritz et al., 1991; Cumming, Wilson, Hall, & Smol, 1995), cultural eutrophication (Battarbee, 1978; Schelske, Conley, Stoermer et al., 1986; Reavie, Hall, & Smol, 1995; Stoermer,

Emmert, Julius, & Schelske, 1996), climate change (Kilham, Theriot, & Fritz, 1996; Saros, Stone, Pederson et al., 2012; Boeff, Strock, & Saros, 2016), and species invasion (Edlund, Taylor, Schelske, & Stoermer, 2000).

It is obvious that planktonic organisms dominate this list; however, an equal or even greater diversity of organisms inhabits the littoral zone of lakes and may similarly utilize dormant propagules. Paleolimnologists recognize that littoral zone diversity is not fully represented in deep-water sediment cores and vice versa. They also recognize that littoral habitats are less suited for paleolimnology because of non-conformable sedimentation, loss of temporal resolution, and higher rates of grazing, resuspension, and mineralization that making littoral habitats less suitable for preserving resting stages (Anderson 2014).

With respect to freshwater organisms that include a period of dormancy in their life cycle, many studies have focused on ecology, physiology, and length of dormancy. From a paleoecological perspective, most of these taxa have also been readily adopted as valuable indicators of ecological change. Research linking dormancy ecology and longevity with paleoecology has been limited to only a few model organisms, primarily the Cladocera *Daphnia*. Few other organisms have been systematically investigated for their potential as models in resurrection ecology, but several offer strong potential because of their prevalence across environmental and temporal scales. One limiting factor to resurrection ecology that can occur in copepods, ostracods, porifera, and dinoflagellates is that the dormant propagules are produced sexually. If there were functional phenotypes selected by the environment, that information could

be lost during the genetic recombination occurring during resting stage formation. This would make the relationships between hatched organisms and historical environmental cues more difficult to detect. A further hindrance can be that viable dormancy propagules cannot not be successfully resuscitated from lake sediments, such as ostracods.

Cyanobacteria and diatoms both produce vegetative resting stages. They will be phenotypically identical to the historic populations and therefore may serve as good organisms for resurrection ecologists. With greater understanding of the phenotypic-environmental relationships, hatching biases, and further paleo-ecological understanding these organisms will likely serve as viable resurrection ecology organisms.

3.4 Diatoms as the Next Model Organism in Resurrection Ecology

With a growing body of literature on paleolimnological indicator values, life histories, phylogenies, dormant stages, and advances in molecular analysis, diatoms are a strong candidate to become a model for resurrection ecology. Diatoms are a diverse group of unicellular or colonial photosynthetic organisms that are unique by possessing an opaline silica cell wall called a frustule (Round et al., 1990). The frustules are intricately ornamented, and when viewed in the light microscope, species level identifications can be readily made. The silica cell walls may persist for millions of years in sediments with a sufficiently high abundance to reconstruct entire life histories (Jewson & Harwood, 2017). With global estimates of over 100,000 species (Mann & Droop, 1996), which occupy

most marine and freshwater habitats, diatoms have proven useful as biological indicators of environmental change among many aquatic systems (Stoermer & Smol, 2010).

Whereas detailed life history and ecological observations for many taxonomic groups of diatoms are wanting, *Aulacoseira* Thwaites is a freshwater planktonic diatom genus that has been well studied. *Aulacoseira* is a species-rich genus and among the most ancestral class of diatoms (Coscinodiscophyceae; Theriot, Cannone, Gutell, & Alverson, 2009). Edgar and Theriot (2004) established phylogenetic relationships of 45 species based on morphology. Diversity in the genus is still being discovered; at least 15 new species have been recently described or transferred (Houk, 2007; Novelo, Tavera, & Ibarra, 2007; Tanaka, Nagumo, & Akiba, 2007; English & Potapova, 2009; Pearce, Cremer, Wagner-Cremer, 2010; Usoltseva & Tsoy, 2010; Tremarin, Luwig, & Torgan, 2012; Van de Vijver, 2012; Tremarin, Paiva, Ludwig, & Torgan, 2013; Tremarin, Ludwig, & Torgan, 2014; Morales, Rivera, Rubin et al., 2015). Given such high species richness, *Aulacoseira* species are found from low-gradient eutrophic (Leland & Porter, 2000) to oligotrophic high-gradient montane rivers (Morales et al., 2015), deep oligotrophic (Jewson & Granin, 2015) to shallow eutrophic lakes (Davey, 1987; Gibson, Anderson, & Haworth, 2003), and even in riverine estuaries (Wang, Li, Lai et al., 2009). *Aulacoseira* spans a latitudinal/temperature gradient from lakes in the tropics (Tremarin et al., 2013) to the northern boreal forests (Fallu, Allaire, & Pienitz, 2000). *Aulacoseira* also appear to be highly adaptable to environmental change; many paleolimnological

studies document shifts in relative abundance of *Aulacoseira* species, but not extirpation, in response to eutrophication and acidification (e.g., Edlund, Engstrom, Triplett et al., 2009).

In addition to biogeographical and ecological characterization, the life histories of several *Aulacoseira* species have been well studied. Jewson (1992) observed the phenology of a population of *Aulacoseira subarctica* (O. Müller) Haworth. He found that *A. subarctica* settled to the lake bottom as dormant cells during the summer and resumed population growth after being resuspended into the plankton during autumn turnover. Once light intensities decreased, sexual reproduction was induced in a small portion of the population during the winter to create annual cohorts. Edlund and Stoermer (1997) characterized this reproductive strategy in diatoms as asynchronous sexuality under good growth conditions. Jewson, Khonder, Rahman and Lowry (1993) observed similar auxosporulation conditions in *A. herzogii*. Jewson (1992) concluded that a full life cycle of an *A. subarctica* cohort could take from 15 to 100 years; however, environmental and physiological controls resulted in a life cycle completed every 4 to 6 years. He also noted that during times of unfavorable conditions dormant stages were common in his study population of *A. subarctica*.

Dormant cells were first documented by Nipkow (1950) in *Melosira islandica* ssp. *helvetica* (now *Aulacoseira helvetica*) and by Lund (1954, 1955) in *M. italica* ssp. *subarctica* (now *A. subarctica*). Resting cells were subsequently reported for *A. granulata* (Sicko-Goad et al., 1986), *A. skvortzowii* (Jewson et al., 2008), and *A. baicalensis* (Jewson, Granin, Zhdarnov et al., 2010; Jewson &

Granin, 2015). Dormant stages appear to have long been present in *Aulacoseira* life history as noted in species from the middle Eocene (Wolfe, Edlund, Sweet, & Creighton, 2006).

Stockner and Lund (1970) were the first to resurrect *Aulacoseira* dormant cells from sediments 11 cm and 15 cm deep in cores taken from several English Lake District lakes. They estimated that dormant cells in the sediments might be viable for up to 50 years. Sicko-Goad, Stoermer, and Fahnenstiel (1986) resuscitated diatoms from sediments and documented changes in cell condition, cellular ultrastructure, and reactivation of chloroplast function during the rejuvenation process. Their experiments confirmed *in situ* observations that aphotic conditions combined with decreasing temperature induced dormant cell formation in *Aulacoseira*. McQuoid, Godhe, and Nordberg (2002) resurrected diatoms from over 37-year-old sediments in a Swedish fjord, laying the foundation for a study of the oldest known diatom resurrection. Härnström, Ellegaard, Andersen, & Godhe (2011) conducted diatom resurrection ecology in from sediments in a Danish fjord. They resurrected 100-year-old *Skeletonema marinoi* resting cells from maritime fjord sediments in Denmark. Dormant cells were resuscitated and cultured for molecular analyses that included sequencing one rRNA and two internal transcriber genes. The greatest genetic difference observed was between open water and fjord samples rather than related to trophic changes within the fjord.

Diatoms have been cultured to answer a wide variety of ecological questions including investigations on cytology (Sicko-Goad, Simmons, Lazinsky

et al., 1988; Schmid, 2001), pesticide inhibition (Guanzan & Nakahara, 2002), reproduction (Basu, Patil, Mapelson et al., 2017), environmental mesocosms (Saros, Michel, Interlandi, & Wolfe, 2005), and ontogeny (Schmid, 1979). Several laboratory culture experiments have been conducted with *Aulacoseira* with resting cell ecology and development. As phosphorus and silica become limiting and cellular growth slows, diatoms respond by increasing their sinking rates (Gibson, 1984) to expedite their journey to the lake sediments and avoid grazing by zooplankton. In culture, Gibson and Foy (1988) found that when silica and phosphorus became limiting, *Aulacoseira subarctica* allocates resources to increasing lipid storage. Gibson and Fitzsimons (1990) found that under aphotic conditions, *A. subarctica* initially utilized carbohydrates followed by lipids and after a month of darkness cells would initiate resting stages. Gibson and Fitzsimons (1991) found that light interruptions of aphotic periods had adverse effects on cell growth. For a riverine population of *A. granulata*, laboratory experiments suggested that resting cell resuscitation was initiated by the presence of another alga *Gloeocystis planctonica* (Poister, Schaefer, Baert et al., 2015).

Recent work with diatom cultures has included the identification of functional parts of the genome. Only a few diatom species have fully sequenced genomes and the genomes appear to be relatively small at 34 Mbp (e.g. *Thalassiosira pseudonana* Hasle & Heimdal; Armburst, Berges, Bowler et al., 2004), 44 to 62 Kbp (e.g. *T. pseudonana*; Armburst et al., 2004, *Seminavis robusta* Danielidis & Mann; Brembu, Winge, Tooming-Klunderud et al., 2014;

Skeletonema marinoi Sarno & Zingone; An, Kim, Noh, & Yang, 2016, *Asterionella formosa* Hassall; Villian, Kojadinovic, Puppo et al., 2017), and 129 to 151 Kbp (e.g. *T. pseudonana;* Armburst et al., 2004; *S. robusta*; Brembu et al., 2014) for the nuclear, mitochondrial, and chloroplast genomes, respectively. The availability of full genomes has allowed researchers to determine gene function using transcriptomics and proteomics studies (Muhseen, Xiong, Chen, & Ge, 2015; Di Dato, Musacchia, Petrosino et al., 2015). For example, in *T. pseudonana*, transcription of genes relating to silica acquisition were up-regulated in preparation for rapid recovery during silica limitation (Shrestha, Tesson, Norden-Krichmar et al., 2012). Furthermore, examination of transcripts suggested that *T. pseudonana* has multiple methods of phosphorus acquisition and allocation to cope with variable concentrations and species of phosphorus (Dyhrman, Jenkins, Rynearson et al., 2012). Nunn, Faux, Hippmann et al. (2013) found cells could switch metabolic pathways to internally recycle nutrients, therefore reducing the effect of environmental resource limitation.

While microorganisms can be difficult to work with, two methods have been developed recently that facilitate culturing and genomics studies on diatoms. The use of serial dilution enables greater ease of extracting and resuscitating dormant resting cells from lake sediments (Piredda, Sarno, Lange et al., 2017). In addition to more efficient culturing of resting cells, single cell isolation and DNA extraction have proven successful for nuclear and chloroplast genomics (Lefebvre, Hamilton, & Pick, 2017; Pinseel, Vanormelingen, Hamilton et al., 2017). PCR amplification of DNA from single cells or dormant eggs could

provide a suitable amount of genetic material from a small amount of source material for paleogenomic studies to forgo resurrection and culturing (Hamilton, Lefebvre, & Bull, 2015; Frisch et al., 2016).

Diatoms are prime candidates to become model organisms for resurrection ecology. Diatoms are globally significant primary producers, form the base of many aquatic foodwebs, and are characterized by high population numbers, diversity, turnover, and longevity in lake sediments, which allows diatoms to respond rapidly to environmental changes and leave highly informative sediment records. And the number of taxa that form dormant cells is large; Sicko-Goad et al. (1989) identified 17 diatom species forming dormant resting cells in the Laurentian Great Lakes. Diatoms have a growing literature on *in situ* ecological and life history studies, are highly adaptable to experimental culturing and relating to paleo-limnological environments (Saros et al., 2005; Jewson, 1992). *Aulacoseira* has a strong foundation in paleolimnological, resting cell, and molecular studies; future transcriptomic and proteomic studies on cell function would benefit their utility in future resurrection ecology studies. Finally, diatoms have a rich history in modern- and paleo-limnological literature combined with the life history trait of dormant cell formation and a growing genomic understanding that can expand their use in resurrection ecology to studying topics such as the effects of eutrophication or climate change on primary producer and primary consumer relationships or rapid evolution within the phytoplankton.

3.5 A Marriage of Necessity

Reiterating the statement by Pelletier, Garant, and Hendry (2009) that "nothing in evolution or ecology makes sense except in the light of the other," we propose that a close collaboration between the fields of paleolimnology and resurrection ecology is important and inevitable. Carefully reconstructed environmental histories of entire lake ecosystems provide a powerful framework to interpret the ecological and evolutionary responses of resurrected organisms in the context of the environment in which they evolved. Similarly, to move paleolimnology beyond a descriptive science and to test ecological and evolutionary hypotheses based on its findings, experimental work is required that can be delivered by resurrection ecology. While studies in resurrection ecology with *Daphnia* have already been fruitful, there is a suite of other organisms ready to be awakened to test predictions across an array of taxonomic diversity. The combination of paleolimnology and resurrection ecology will be key to empirically provide answers to questions surrounding the capacity of organisms to adapt to rapid environmental change, one of the most pertinent problems of our planet today.

3.5.1 Evolutionary Responses to Environmental Change

The loss of species from lakes is often documented in paleolimnology but is less commonly approached via resurrection ecology. There are hundreds of paleolimnological studies that apply correlative analyses to document and hypothesize why aquatic species become locally extirpated. For example, Stoermer, Wolin, Schelske, and Conley (1985) documented the local extirpation of the deep chlorophyll layer diatom community (numerous *Cyclotella* diatom

species) following post-European eutrophication of Lake Ontario. The combined effects of light and silica limitation following enhanced nutrient loading resulted in the loss of this characteristic diatom community from the lake.

Examples of species extirpation studied with resurrection ecology are less numerous. By analyzing ephippia in sediment cores, Hairston et al. (1999) discovered that *Daphnia exilis* had been introduced into Lake Onondaga in the 1920s and persisted into the 1980s. Based on genetic analysis of resurrected populations from the 1970s and 1980s, it was determined that a single genotype had been introduced into the lake, and that at the time of its local extirpation the population had remarkably low genetic diversity that may have contributed to population demise.

Whether a species is lost from a habitat is determined by their adaptive capacity; does their phenotypic plasticity allow them to respond to changing abiotic and biotic pressures, or if not, can they disperse, diapause or more rapidly evolve simultaneous with environmental change (Reed, Schindler, & Waples, 2011)? Adaptive capacity is one way to examine an organism's ability to change niche space (Chevin, Lande, & Mace, 2010; Beever, O'Leary, Mengelt et al., 2015). The adaptive capacity model estimates a species' chance of survival or extinction by evaluating the costs of phenotypic plasticity and overall genetic variance against the magnitude and duration of an environmental stressor. The rate of environmental change and species adaptation is key in determining the potential for survivability (Hairston, Ellner, Gerber et al., 2005). To understand the adaptability of species and to improve niche models, species resurrection

must be coupled with a detailed knowledge of specific environmental change on long time scales in natural settings, a deliverable product of paleolimnological research. This will ultimately bring a more complete understanding of how current environmental pressures might affect biodiversity and evolution (Franks, Hamann, Weis, 2018).

3.5.2 Eco-evolutionary Dynamics of Biological Communities

While understanding how a single species might adapt to environmental perturbation, it is also important to understand how the interplay of community responses can affect evolutionary dynamics. Community level responses have often been observed in paleolimnology. The abundance of diatom and chironomid assemblages has been used to reconstruct Holocene climate changes (Reinemann, Princhu, Bloom et al., 2009). Also, diatoms, chrysopytes, and cladocerans have been used to reconstruct lake acidity (Arseneau, Driscoll, Brager et al., 2011). However, the interplay among member of these paleo-communities and their response to the environment as a community has not been directly assessed, and the drivers of population change may be more complicated than expected (Becks, Ellner, Jones, & Hairston, 2012; Kinnison et al., 2015).

In some cases, eco-evolution can be more strongly driven by interspecies evolution rather than environmental change or resource availability, as shown in predator-prey relationships (Becks et al., 2012). Evolutionary feedback loops can exist that are not readily observed in assemblage data (Kinnison et al., 2015). Dormant propagules in lake sediments offer the unique ability to use natural,

long-term community records whose propagules can be resuscitated for experimentation to understand models of community adaptation and eco-evolutionary dynamics.

3.5.3 A Mechanistic Approach Links Paleoecology and Resurrection Ecology

In paleolimnology one often unspoken and untested assumption in the interpretation of sediment records is that the ecology of organisms was the same in the past as it is today. We interpret the ecology of the past using our knowledge of modern ecology, which rarely accounts for species adaptation. For example, Stoermer, Emmert, and Schelske (1989) documented size decrease in the diatom *Stephanodiscus niagarae* during cultural eutrophication in Lake Ontario and suggested that the diatom might be succumbing to sexual failure. However, Edlund and Stoermer (1991) showed that small-celled populations of *S*. *niagarae* from eutrophic areas of the Great Lakes were perfectly capable of undergoing sexual reproduction, a phenotypic life history adaptation to nutrient enrichment. While most paleolimnologists examine only sediment records of species response and change, recent studies link historical species response to modern experimental species responses (Saros, 2009). Saros et al. (2005) conducted experiments relating the response of modern diatom species to nutrient supply and then interpreted the historical response of the same taxa in alpine lakes using the experimental results. Mechanistic studies have also been applied to resurrection ecology with the help of molecular tools. Common garden experiments (Kerfoot & Weider, 2004) have been used on resurrected *Daphnia*

to genetically characterize historical populations as they adapted to predation. Roy Chowdhury et al. (2015) used resurrected *Daphnia* from a eutrophic Minnesota lake to study adaptation using a transcriptomics approach to compare gene expression under different food quality scenarios. As we expand the database of diatom genome sequences, changes in genes and proteins can be related to cell functionality to better understand historical species response (Davis, Shaw, Etterson et al., 2005).

3.5.4 Paleoecology, Resurrection Ecology and Restoration Ecology

The resuscitation of dormant propagules from the sediment "seed bank" may allow organisms specific to each lake to recolonize under restored conditions and could prove useful for re-establishing the lower trophic levels during restoration efforts and help preserve the Earth's remaining biodiversity. Ecological monitoring and environmental policies in the late 20th century have led to the rehabilitation of some lakes from effects of acidification (Lotter, 2001; Stoddard, Jefferies, Lükewille et al., 1999) and cultural eutrophication (Antoniades, Michelutti, Quinlan et al., 2011). However, after a successful return to historical water quality conditions, a novel ecosystem usually results, that is, communities do not return to their historical makeup (Lotter, 2001). In part, restoration efforts may be confounded by alternative or multiple environmental drivers such as climate change (Battarbee, Morely, Bennion, et al., 2012, Sivarajah, Rühland, & Smol, 2017) resulting in extended period to recovery or a moving baseline for restoration targets (Battarbee, Anderson, Jeppesen, & Leavitt, 2005; Bennion, Batterbee, Sayer, et al., 2011). Alternatively, modern

populations may not have the adaptive capacity to reorganize to similar historical communities. One common practice in ecological restoration is the facilitated recolonization by similar communities, ideally with similar phenotypes (Buisson, Alvarado, Stardic, & Morellato, 2017). Whereas plant (Ozimek, Gulati, & van Donk, 1990) and fish communities (Volta, Yan, and Gunn, 2016; Søndergaad, Lauridsen, Johansson et al., 2017) are commonly the focus of restoration efforts, communities that produce dormant propagules such as zooplankton, phytoplankton, and bacterial communities, are often neglected. While bio-manipulation has often yielded unintended consequences, suggested strategies for facilitating de-extinction in terrestrial ecosystems can be applied to successful restoration of extirpated aquatic taxa. The resting stages of phytoplankton can be viewed as a temporal genotypic refuge of an ecosystem waiting a return to pre-disturbance conditions (Ellegaard, Godhe, & Ribeiro, 2018). Paleoecological data can help ecologist choose which taxa are appropriate for restored conditions (Wood, Perry, & Wilmshurst, 2017). With the ability to hatch, culture, and experiment with historical organisms from dormant propagules in lake sediments, resurrection ecology and paleolimnology allow insight into appropriate restoration goals and how a restored community might behave.

3.5.5 The Challenges of Marriage

The fields of paleoecology and resurrection ecology each have their own limitations that they bring to their marriage. Paleolimnologists often presume that a single core taken from a central deep basin provides all the information required to understand the ecological history of a lake. Biases associated with

sediment cores and depositional environments are common and may include unconformable and hiatuses in sedimentation, violation of dating model assumptions, bioturbation, down-core degradation and absence or loss of proxies, temporal resolution of sediment slices (i.e., details needed from each core slice vs what is the period represented by that slice), and spatial biases in core records (e.g., littoral vs profundal). Paleolimnologists are encouraged to exercise appropriate cautions in coring, analyses, and interpretation (Battarbee et al. 2012) when considering temporal and spatial scaling of lake and landscape changes (Anderson 2014).

For resurrection ecologists one of the greatest limitations is the reduction in egg viability with sediment age, and furthermore we do not know how well the laboratory hatched propagules are representative of the overall diversity or dominant genotypes or phenotypes of the sampling period (Weis, 2018). In addition to the stratigraphic and geomorphic variability, we do not know how physical, chemical, or other limnological processes effect dormant propagule burial and preservation.

3.6 Conclusions

Lake sediments preserve unparalleled historical archives of organismal and environmental change. Paleoecologists use the power of multi-proxy and multi-lake analyses to reconstruct precise records of historical change at lake, watershed, and global scales. New directions in paleolimnology are melding experimental neo- and paleo-limnological approaches to better understand historical and future species and community response to

environmental change. Resurrection ecologists rely on the “egg bank” of viable dormant propagules in lake sediments to test the capacity of organisms to adapt to our rapidly changing environment using *Daphnia* as the model organism. However, numerous aquatic organisms that are cornerstones in paleoecology (e.g. diatoms, cyanobacteria) also produce dormant propagules that offer new opportunities to test species and community adaptation, study eco-evolutionary feedback and co-evolution of primary producers and consumers, discern mechanisms of species response, and fuel the burgeoning collaboration between these two fields.

Chapter 4

A multi-proxy approach to assessing the response of diatoms to aeolian dust enrichment in the recent history of Cedar Bog Lake, Minnesota

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Abstract

Dust-driven enrichment of aquatic ecosystems can have wide ranging ecological impacts, including in lakes that are remote and nutrient-limited. By selecting a lake with a small peat-bog watershed, which is generally mineral poor, we used paleolimnological evidence to evaluate the ecological impacts of dust deposition to Cedar Bog Lake, Minnesota. After two centuries of relatively stable geochemistry, increases in inorganic matter and dry mass accumulation rates coincided with agriculturalization of the Great Plains and the Dust Bowl during the first half of the 20th century. The geochemical increases were followed by increases in the flux of organic matter, diatoms, and sedDNA indicating elevated lake productivity. Biogenic Si and DNA were useful in detecting ancient communities in the absence of microfossils. Here we demonstrate that during a period of dust enrichment, Cedar Bog Lake increased in primary productivity as seen through the diatom community and organic matter accumulation. Corresponding to regional declines in dust deposition, the diatom community responded to lower nutrient inputs with further shifts in species abundances toward the end of the 20th century.

4.1 Introduction

Anthropogenic dust deposition has significantly increased recently (Neff et al. 2008) affecting lakes far distant from the dust sources (Brahney et al. 2019). Anthropogenic dust can alter the chemistry of lakes (Ballantyne et al. 2011, Zhu et al. 2019) and change the ecology of lakes (Brahney et al. 2014, 2015a, b, Spaulding et al. 2015). Dust deposition and its ability to overcome growth limitations by the macro- and micro-nutrients P, Fe, N, and Si, which are key for algae and diatom productivity, are predicted to be geographically dependent on dust source (Moore et al. 2013, Bhattachan et a. 2016, Mahowald et al. 2017, Hettiarachchi et al. 2020). In order to examine the role of atmospheric deposition as a vector of increased sedimentation and nutrient deposition in Minnesota lakes, Engstrom et al. (2019) deployed dust collectors and conducted paleolimnological investigations in five lakes with low to no mineral input from their small peatbog watersheds. The dust collectors captured aeolian mineral matter at rates which were reflective of 1/10 of the lake sedimentation rates; additionally, the modern dust deposition was found to contain high concentrations of P. The significant 20th century increases in mineral sedimentation rates measured were consistent with similar changes observed in wilderness lakes (Dietz et al. 2015). Wanting in many studies on dust deposition is assessment of the ecological effects of increased dust deposition on lakes; this aspect can be investigated using biological paleolimnological proxies.

Nutrient enrichment, even subtle increases (Justus et al. 2020), can alter biological assemblages in aquatic ecosystems (Paerl and Otten 2013, Hobbs et al. 2016). Diatoms have proved to be excellent indicators of a wide variety of

environmental change including nutrient enrichment (Bennion et al. 1996, Potapova and Charles 2007). In particular, diatoms show strong responses to P and Si enrichment through assemblage changes (Anderson et al. 1990) and increased abundance (Conley et al. 1993). Si a critical element for diatom productivity as it is used in the construction of their highly ornate cell walls called frustules (Round et al. 1990). Characterization of diatom assemblages relies primarily on morphological identification of the frustules and enumerating the diatoms with microscope-based quantitative methods (Battarbee 1973, Scherer 1994). Diatom frustules normally persist in lake sediment records and have been used to reconstruct biogeochemical changes to lakes such as acidity (Hall and Smol 1996) and nutrient enrichment (Laird et al. 2017). Furthermore, the Si content of the frustules, measured as biogenic Si (BSi), has been used as a geochemical proxy for diatom abundance (Conley and Schelske 2002).

Recently, environmental assessment and detection of diatoms have branched into environmental genomics (eDNA), where fragments of eDNA are sequenced and matched with reference libraries or observed in a taxonomy-free method to describe the biological community (Vasselon et al. 2017a, Smucker et al. 2020). These methods are being developed for diatoms in modern and historical lake sediments (Stoof-Leichsenring et al. 2015, 2020a). Similar to light microscopic (LM) enumeration (Lee et al. 2019, Tyree et al. 2020) there are uncertainties in DNA quantification (Vasselon et al. 2017c, Vasselon et al. 2019) and taxonomic consistency (Zimmerman et al. 2014, Rimet et al. 2019). DNA relative read copies produce both similarities and differences with LM

enumeration, e.g., tracking ecological changes for certain genera like *Aulacoseira* or *Staurosira*, whereas the genus *Tabellaria* is best detected using the microscope (Stoof-Leichsenring et al. 2020). Further hindering direct comparisons, genomic content is related to cell volume, wherein larger cells contain more DNA and therefore produce a disproportionate number of reads compared to smaller bio-volume taxa (Vasselon et al. 2019). Comparisons of diatom quantification methods and leveraging the advantages of each – geochemistry, LM, and DNA – should provide greater insight into environmental changes in abundance and inferred productivity by combining the methods.

Cedar Bog Lake, located within the Cedar Creek Ecosystem Science Reserve near East Bethel, Minnesota, U.S.A. is part of a long-term ecological research site. The reserve has hosted a wide variety of ecological research since the 1930s (Deters 1931), including foundation ecology on resource limitation and community structure (Tilman 1982). Originally formed by remnant glacial ice block melt, Lindeman (1941) described Cedar Bog Lake as a senescent lake expected to have only 250 more years until completely infilling to become a bog. Critical to our study is the extensive bog mat that surrounds Cedar Bog Lake and captures eroded mineral matter from the watershed; all mineral inputs should be primarily atmospheric or represent in-lake production (diatom silica). In addition to characterizing the geomorphic history of Cedar Bog Lake, Lindeman observed that the water levels in the lake were directly tied to precipitation in a positive relationship and furthermore the lake levels had a direct impact on the littoral emergent vegetation community composition. Building on this fundamental

understanding of Cedar Bog Lake's limnology, Lindeman (1942) went on to describe the seasonal community change and flow of energy between organisms in what became known as trophic levels. Further building upon this foundational work and Engstrom et al. (2019), we leverage Cedar Bog Lake and its ability to respond to atmospheric deposition and use the lake sediment record to: 1) examine the lake's historical ecological response using BSi, 2) quantify historical diatom community structure using morphological and DNA enumeration, 3) compare BSi, morphological count density, and DNA read copies counts as proxies for diatom growth and response, and 4) link changes in the diatom paleolimnological proxies to changes in dust deposition.

4.2 Methods

4.2.1 *Sampling, subsampling, dating and geochemistry*

On 17 May 2016, a sediment core (~100 cm in length) was collected from Cedar Bog Lake using a surface piston corer operated from a boat (Wright 1991). The core was sectioned ashore at 2-cm intervals, and samples were stored under refrigeration in polypropylene jars until being analyzed. Sediment chronology was determined using ^{210}Pb and ^{137}Cs dating, where core dates and sedimentation rates were calculated using the constant rate of supply model (Appleby and Oldfield 1978) with dating uncertainty determined by first-order propagation of analytical counting error (Binford 1990). Bulk-density (dry mass per volume of fresh sediment), inorganic content, organic content, and carbonate content of sediments were determined using loss-on-ignition (LOI) techniques (Dean 1974). Further details on sample collection, LOI, and establishment of the

^{210}Pb chronology can be found in Engstrom et al. (2019) or previously in Chapter 2.2.

4.2.2 *Diatom preparation and analysis*

Biogenic silica (BSi) was measured with a wet-alkaline digestion method using weighed subsamples (~30 mg) extracted with dilute $NaCO_3$ (DeMaster 1979, Conley and Schelske 2002). Dissolved silica was measured colorimetrically on a Unity Scientific SmartChem 170 discrete analyzer as molybdate reactive silica (SmartChem 2012a).

For light microscopy, diatoms were prepared by placing approximately 30 mg freeze-dried core material in a 50 ml polycarbonate centrifuge tube and adding 2-5 drops of 10% v/v HCl solution to dissolve carbonates. Organic material was subsequently oxidized by adding 10 ml of 30% H_2O_2 and heating for 3 hr in an 85°C water bath. After cooling the samples were centrifuged and rinsed six times with deionized water to remove oxidation byproducts. Material was then transferred to Battarbee chambers where cleaned material dried onto coverslips (Battarbee 1973). Coverslips were permanently attached to microscope slides using Zrax mounting medium (Ramstack et al. 2008). Diatoms were identified on an Olympus BX-50 light microscope along measured random transects to the lowest taxonomic level under 1000-1250X magnification (full immersion optics of NA>1.3). 600 valves were enumerated in each sample using the voucher flora method (Bishop et al. 2017). Valve density, a measure of overall diatom abundance, was calculated using a modification to the equation provided by Scherer (1994) where the area of the bottom of the beaker was

replaced by the area of the Battarbee settling chamber. The Scherer (1994) equation $T = (NB/AF)/M$ was used where T is the number of microfossils per unit mass, N is the total number of microfossils counted, B is the bottom area of the Battarbee chamber (mm^2), A is modified for the diameter per field of view (mm), F is modified for the length of the transect (mm), and M is the mass of the sample (g). The resulting density of diatoms was then multiplied by the DMAR to estimate the historical flux of diatoms. Diatom counts were also converted to percentage by taxon; abundances are reported relative to total diatom counts in each sample.

4.2.3 *DNA extraction, amplification, sequencing and taxonomic assignment*

Sediment DNA was analyzed at every sediment increment (n=49) in triplicate; furthermore, the two top-most core increments were subsampled in triplicate and subsequently processed in triplicate. Frozen samples were thawed for 1-2 hours before processing. Under a laminar flow hood, sterile cotton swabs (Fisher, cat# 22-363-173) were coated with sediment matter, and the swabs were placed in the corresponding extraction plate or tube. Sterile tweezers and pliers were used to handle cotton swabs and remove the wooden ends of the cotton swab before extraction. Plates or tubes were immediately processed or stored in -20°C until the extraction process could be performed. Sample barcodes were recorded and assigned a well within the 96-well plate or numbered extraction tube. A MoBio PowerSoil htp-96 well Isolation Kit (Cat#12955-4) was used according to the manufacturer's protocol to extract genomic material from sediment samples. Genomic DNA was eluted into 100 μl and frozen at -20°C.

For amplicon sequencing the 23S ribosomal subunit was targeted for its nucleotide variability, which can be useful in taxonomic delineation (Sherwood & Presting 2007, Cannon et al. 2016), including for diatoms (Hamsher et al. 2011). Amplicons were generated through polymerase chain reaction from each genomic DNA sample using the Diam23Sr1 23S primers (3': GGACAGAAAGACCCTATGAA, 5': TGAGTGACGGCCTTTCCACT). Each 40 *µl* PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5 *µ*l Master Mix, 0.5 *µ*M of each primer, 1.0 *µ*l of gDNA, and 10.5 *µ*l DNase/RNase-free H2O. The DNA was PCR amplified using the following conditions: initial denaturation at 94 °C for 3 minutes, followed by 40 cycles of 30 seconds at 94 °C, 45 seconds at 55 °C, and 1 minute at 72 °C, and a final elongation at 72 °C for 10 minutes. To determine amplicon size and PCR efficiency, each reaction was visually inspected using a 2% agarose gel with 5 *µ*l of each sample as input. Amplicons were then cleaned by incubating amplicons with Exo1/SAP for 30 minutes at 37 °C following by inactivation at 95 °C for 5 minutes and stored at -20°C. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 *µ*M of each primer and 2 *µ*l of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 sec, 55 °C for 30 seconds and 72 °C for 30 seconds. Five *µ*l of indexing PCR product of each sample were visualized on a 2% agarose gel to ensure the success of the barcoding PCR.

Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25 μl of PCR amplicon was purified and normalized using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples were then pooled together by adding 5 μl of each normalized sample to the pool. Sample library pools were sent for sequencing on an Illumina MiSeq (San Diego, CA) in the University of Colorado Boulder BioFrontiers Sequencing Center using the v2 500-cycle kit (cat# MS-102-2003).

Sequences were demultiplexed by taking advantage of Golay barcodes via QIIME v1.9.1 (Caporaso et al. 2010). The following options were used to output raw unfiltered fastq files for both forward and reverse reads: split_libraries_fastq.py-q 0 --max_bad_run_length 250 --min_per_read_length_fraction 0.0001--sequence_max_n 250 --store_demultiplexed_fastq. Forward and reverse read sequences were trimmed to 235 nucleotides via the usearch8 option -fastx_truncate. Sequences were then merged using the -fastq_mergepairs option in usearch8 (Edgar 2010). The forward primer (5'- GGACAGAAAGACCCTATGAA -3') and reverse primer (5'-TGAGTGACGGCCTTTCCACT -3') were removed using cutadapt (Martin 2011). From here forward, the 23S amplicons were processed via the UPARSE pipeline (Edgar 2013) and taxonomy was assigned via the SINTAX protocol (http://www.drive5.com/usearch/manual/utax_user_train.html) available in usearch (v8.1.1861) (Edgar 2010). Specifically, sequences were quality trimmed to have a maximum expected number of errors per read of less than 0.5 and

OTUs were clustered at 97% similarity with de novo chimera checking enabled. To assign taxonomy to each OTU, an `in-house` SINTAX 23S reference database was constructed by downloading any annotated GenBank (Benson et al. 2005) records that contain the 23S gene. All resulting 23S amplicon regions were dereplicated to 100% sequence identity and any identical sequence across lineages were collapsed to the lowest-common-ancestor. Closed reference OTUs were generated by searching against the 23S reference database at 97% sequence similarity. To ensure increased specificity of 23S OTU assignment against the reference database the -maxaccepts and –maxrejects search options were set to 0. Abundances of OTUs were reported by the number of sequence reads per sample and converted to relative abundance by dividing the number of reads for each OTU by the total number of reads for each sample.

4.2.4 *Data analysis*

Total DNA and total diatom DNA as number of sequence reads, dry mass accumulation rates, geochemical percentages and constituent fluxes were plotted in stratigraphic plots to show changes over time. A Pearson's correlation matrix was constructed to examine significant trends among these constituents and with time. Diatom relative reads and microscope enumerated relative abundances were first square root transformation in R using the Hellinger method in the Vegan package (Legendre and Gallagher 2001, R Core Team 2014, Oksanen et al. 2013). Stratigraphy of predominant diatoms (species with greater than or equal to 3% relative abundance in one or more core depths) was plotted against core date using the *Rioja* R package (Juggins and Juggins 2019).

Temporal relationships among the dominant diatom communities within each sediment core layer were explored using the Euclidian distance matrices in a constrained cluster analysis (CONISS). Significant cluster groups were determined using the broken stick model (MacArthur 1957).

4.3 Results

4.3.1 *Geochemistry*

Lake sediments were dated by ^{210}Pb to a depth of 78 cm (1805 ± 17 yr) and date estimates were extrapolated to a depth of 1 m, which was estimated to represent ~1700 C.E. (Table 4.1, Supplemental Table 4.1). The dry mass accumulation rate was on average 0.016 g cm^{-2} yr^{-1} with a maximum of 0.028 g cm^{-2} yr^{-1} (2015 C.E.) and a minimum of 0.007 g cm^{-2} yr^{-1} (1830 C.E.). The dry weight percentage of organic matter averaged 67% and was the largest sediment constituent; inorganic and $CaCO_3$ averaged 29% and 3%, respectively. The BSi was on average 4%, ranging from 1.2% (1760 C.E.) to 5.8% (1985 C.E.). The dry mass accumulation rate and the percentage and flux of inorganic materials, carbonates, and BSi increased significantly over the 20th century (Figures 4.1, 4.2). The flux of organic matter also increased at the same time, whereas the percentage of organic matter decreased to 53%, having composed the dominant sediment component during the 18th and 19th centuries, around 75%.

There were significant relationships among the geochemical parameters in addition to over time (Figure 4.2). Dry mass accumulation rates, calcium carbonate percentage and flux, inorganic sediment percentage and flux, biogenic silica percentage and flux, and organic sediment flux were all positively

correlated with each other and time ($p < 0.05$). The percentage of organic sediment was negatively correlated with the other biogeochemical parameters and time ($p < 0.05$).

4.3.2 *Light Microscopy*

We enumerated 3,364 diatom valves representing 46 morphological species from 13 sediment depths using light microscopy (Supplemental Figure 4.1). Based on statistical abundance criteria (>3% abundance and >3 samples), 29 taxa were included in stratigraphic analysis. Few diatom microfossils were found in sediments deposited prior to 1910 or sediment core intervals below 49 cm; after considerable effort, only 195 valves could be enumerated below 49 cm. From 1910 to the present, only a single significant change was observed in the diatom assemblage, and that occurred in the late 20th century between 1980 and 1995 (Figure 4.3). The assemblage transition involved a shift from AMA01 (*Amphora ovalis*), AUL01 (*Aulacoseira cf. italica*), AUL03 (*Aulacoseira ambigua*), and COC01 (*Cocconeis placentula*) to an increase in EUN01 (*Eunotia formica*), GOM04 (*Gomphonema sp*. 4), GOM06 (*Gomphonema truncatum*), GOM09 (*Gomphonema sp*. 9), SEL02 (*Sellaphora sp*. 2), SEL03 (*Sellaphora pupula*), SYN01 (*Ulnaria delicatissima*), ULN01 (*Ulnaria sp*. 1), and ULN03 (*Ulnaria ulna*) (Table 4.2, Supplemental Figure 4.1). The accumulation rate of valves (diatom flux) was highly variable throughout the 20th century (Figure 4.1).

4.3.3 *Diatom DNA*

From the DNA amplification, sequencing, and bioinformatics pipeline, 4,453 23S exact sequence variants (ESVs) composed of 2,328,750 sequences

were recovered. Total recovered reads of all DNA were steady, without much fluctuation, during the 18th and 19th centuries and then declined over the 20th century. whereas the reads of diatom DNA increased over the 20th century (Figure 4.1). The flux of both total DNA and diatom DNA showed increases over the 20th century.

From the 445 diatom ESVs detected, 31 were found to occur within our statistical abundance criteria (>3% abundance and >3 samples). Constrained cluster analysis divided the diatom assemblages detected using DNA in the mid-19th century (ca. 1865), after which ESVs 031283 (*Sellaphora sp.)*, 031852 (*Sellaphora sp.*), and 009072 (*Sellaphora pupula*) became predominant (Figure 4.4, Table 4.3). Near the top of the sediment core, ESVs 034468 (*Asterionella formosa*), 065006, 006396 (*Navicula sp.*), 059585 (*Sellaphora sp.*), 037041, 065127, and 062081, became more abundant; these ESVs were not detected earlier in the paleorecord. The ESVs 031370, 032448, 001771, 000050 (*Thalassiosira*), and 00009 (*Thalassiosira rotula*) were detected prior to the mid-19th century, but with few or no detections after the 1865 break. Sediment levels dated to 1853, 1845, 1812, 1803, 1794, 1784, 1766, 1756, 1746, 1725, 1715, and 1704 did not yield any recoverable diatom DNA.

4.4 Discussion

The Cedar Bog Lake paleo-sediment record revealed two centuries of uniform geochemistry (1700 to 1900 C.E.), followed by a rapid increase in sedimentation rates during the 20th century. The increased sedimentation was largely driven by an increase in the inorganic sediment content and

accumulation. At the same time, the percentage of organic matter in the sediment declined, although the organic accumulation rate greatly increased with the overall increases in DMAR. Like the trends in organic matter, the total reads of DNA were relatively unchanged over the 18th and 19th centuries, with the reads declining over the 20th century. In contrast, the flux of DNA reads during the 20th century shows an increase in genomic material deposition compared to the previous centuries.

The increased flux of organic matter and DNA is coincident with increases in content and accumulation of inorganic matter. Because watershed inputs of mineral matter to Cedar Bog Lake are minimal, increases in inorganic matter content and flux reflect changes in either atmospheric deposition directly to the lake or mineral matter produced within the lake, such as biogenic silica. While inorganic matter increased from approximately 18% to over 40% by weight during the 20th century, biogenic silica increases only accounted for 1% of inorganic content in the 18th and 19th centuries to as high as 6% in the 20th century. Engstrom et al. (2019) contends that the only likely source of increased inorganics (non-biogenic silica) must be atmospheric and related to dust deposition.

North America dust deposition increased over the 20th century (Neff et al. 2008, Ballantyne et al. 2011). Dust is shown to enrich lakes with phosphorus (Brahney et al. (2014) and carbonate (Clow et al. 2016) in the western United States, and the ecological impacts need to be further understood (Brahney et al. 2015). Forest soil chemistry (e.g., Ca, Mg, K, Na, and P) is consistent with the

nutrient composition of dust deposition across Minnesota, Wisconsin, and Michigan, illustrating a significant landscape biogeochemical influence (Grigal and Ohmann 1989). Gordon and Todhunter (1998) found that dust delivery in western Minnesota primarily occurred in winter and spring as low magnitude events. However, dust deposition was shown to be decreasing in frequency during the last half of the 20th century coincident with improved soil conservation measures (Todhunter and Cihacek 1999). We contend that the history of increased dust deposition (Engstrom et al. 2019) and the nutrients it delivered fundamentally altered the 20th century ecology of Cedar Bog Lake.

The increase in lake productivity is echoed by multiple proxies for diatom growth. Sediment BSi was very low in Cedar Bog Lake through the late 18th century, but rapidly increased over 2-fold, coincident with the 20th century influx of inorganic materials. This change is similarly reflected by increased abundance and better preservation of diatom microfossils, which were not readily preserved prior to the 20th century. The diatom community as represented by 23S exact sequence variants show an increase in sequence read copies during the first half of the 20th century. While dust deposited to Cedar Bog Lake is shown to carry phosphorus (Engstrom et al. 2019), which could spur diatom growth, diatoms can also be limited in Si which has been shown to be a large component of dust (Tegan and Kohfeld 2012). Silica limitation could explain the low diatom count and BSi values prior to the 20th century either through Si dissolution (Flower 1993, Ryves et al. 2001) or limiting diatom productivity (Conley et al. 1993). In the absence of quantifiable diatom microfossils and BSi, bulk sediment DNA

reads proved useful in providing evidence for the presence, abundance, and assemblage composition of diatoms back to the mid 18th century.

The effectiveness of a target amplicon region to detect diversity is dependent on two factors: genomic variability for a given organismal group and relating the reads to taxonomic information within on the breadth and coverage of a reference database (Zimmerman et al. 2014, Rimet et al. 2019). The 23S amplicon region has been used to effectively delineate diatom species (Sherwood and Preston 2010, Hamsher et al. 2011, Gou et al. 2015, Liu et al. 2020). Differences can arise when trying to compare diatom abundance between microfossils and DNA copies (Vasselon et al. 2017c, 2019). In light of that evidence, we consider some of the shortcomings observed here for the taxonomy of dominant 23S ESVs defined in GenBank (Benson et al. 2005). Two conspicuous 23S taxonomic returns were the detection of *Melosira tropica* and *Thalassiosira rotula*, the prior named for its occurrence in tropical habitats (Crouan 1870) and the latter a planktonic marine species (Syvertsen 1977). In the Cedar Bog Lake sediment core, the dominant planktonic diatoms observed in light microscopy were of the genus *Aulacoseira*, a closely related genus containing many taxa separated from *Melosira.* More puzzling are the observations of *Thalassiosira* which is closely related to freshwater genus *Stephanodiscus*, however there were no microscopic detections of *Stephanodiscus*. These discrepancies highlight areas where genomic biomonitoring can be optimized through better understanding of 23S amplicon

genomic variability within the diatoms (Gou et al. 2015) and reference database development (Ruppert et al. 2019).

While the density of valves, accumulation of diatom reads, and BSi content and accumulation—together representing increased growth of diatoms—coincide with the early 20th century shift in Cedar Bog Lake's geochemistry, diatom assemblages did not show any dramatic changes in the early 20th century. The light microscopic record indicated an ecological change in the lake's more recent post-1980s history. This shift was characterized by a loss of planktonic *Aulacoseira* to benthic *Eunotia*, *Gomphonema,* and *Sellaphora* species, suggesting a likely increase in water clarity (Liu et al. 2020) less lake mixing (Gibson et al. 2003), or lower limiting nutrients in the water column. This change may be a product of lake recovery from initial atmospheric loading since the peak of dust deposition in the 1970s (Engstrom et al. 2019, Schindler 1974, Gordon and Todhunter 1998) or increased macrophyte abundance (Lindeman 1941, Brugam et al. 1998, Lane et al. 2007).

The 20th century dust-driven nutrient enrichment is not unique to Cedar Bog Lake; anthropogenic dust deposition, dust-driven productivity, and altered community structure have been observed for lakes in other regions of the world (Morales-Baquero et al. 2006, Jiménez et al. 2018). Dust deposition rates into North American lakes have shown increases and declines over the 20th century, attributed to early 20th century drought and later implementation of soil conservation practices (Todhunter and Cihacek 1999, Neff et al. 2008). Similar to western North American lakes (Brahney et al. 2014, 2015), the Cedar Bog Lake

sediment core captures the ecological effects of increased productivity by dust enrichment to a lake in central North America. Furthermore, as regional dust records have described a recent decreasing trend in dust deposition rates (Gordon and Todhunter 1998), the diatom microfossil record and decreasing inorganic matter suggests an improvement in water clarity of Cedar Bog Lake during the end of the 20th century. The findings presented here for Cedar Bog Lake highlight the potential ecological impacts of dust enrichment to lakes with nutrient-limited and low mineral content watersheds.

From his careful and dedicated work in the late 1930s, Raymond Lindeman showed the hydrologic reliance of Cedar Bog Lake on atmospheric inputs (Lindeman 1941). In one of the first ecosystem characterizations of energy transfer, Lindeman and his wife Elanor (Sterner 2012) observed a phyoplankton dominance of *Melosira* and *Fragilaria* (Lindeman 1942). Presumably since *Melosira* is the historical taxonomic placement for *Aulacoseira,* we observe the same dominant phytoplankton in the Cedar Bog Lake paleolimnological record. Furthermore, with our paleolimnological interpretations of dust enrichment to Cedar Bog Lake, we conclude that Lindeman's associations between trophic levels were made during a time of ecosystem enrichment.

4.5 Conclusions

We show that aeolian enrichment likely stimulated lake productivity in Cedar Bog Lake, especially for the diatoms. Furthermore, multiple approaches to assessing diatom-productivity provided complementary lines of evidence. The 23S amplicon was useful in detecting the presence of diatoms and identification

for some taxa. However, the results also characterized some (unlikely) marine diatoms, so there is need to bolster 23S libraries with more freshwater diatom taxa. Recent changes to the diatom ecology and the reduction in mineral matter input to Cedar Bog Lake suggest ecological recovery from dust enrichment, which coincides with regional observations of declines in dust deposition. A modern analysis of Cedar Bog Lake using Lindeman's "food-cycle" or trophic-dynamic model would likely confirm an altered trophic status for Cedar Bog Lake by comparing the lake during dust enrichment (historical) and post-recovery (modern).

Chapter 5

Reconstructing historical change in lake ecology using metabarcoding: Using bulk sediment sequencing to uncover environmental shifts in Minnesota's Hill and South Center lakes over the past 200 years

Abstract

Community reconstructions of aquatic organisms in lakes using bulk sediment or environmental DNA (eDNA) have become more common in the recent decade. Improvements to the delineation of taxa and availability of reference databases allow for implementation of highly diverse biological surveys. Here we demonstrate that in Minnesota; 1) eDNA can be recovered from modern and historical lake sediments, 2) amplicon sequence variants, especially *rbcL*, can provide spatio-temporal environmental trends in planktonic diatom communities, and 3) diatom microfossils and ancient eDNA fragments can be combined in tandem to reconstruct historical changes. While taxonomy-free amplicon sequence variants can provide meaningful ecological inferences, establishing local taxonomic reference databases will provide a crucial link to connect the past century of diatom limnological research in Minnesota with a promising future.

5.1 Introduction

Analysis of extracellular DNA or environmental DNA is an increasingly refined and adopted method for collecting aquatic biological data. These surveys can be used as a cost-effective means for wide-scale ecosystem health

monitoring (Kelly et al. 2018, Bailet et al. 2019), species invasion (Holman et al. 2019), or to inform eco-evolutionary relationships in changing environments (Stoof-Leichsenring et al. 2015). While much work is needed to equate traditional and molecular derived species abundances, metabarcoding of environmental DNA samples can sometimes reveal hidden diversity not apparent in traditional identification methods (Shirouzu et al. 2020). In environmental assessment, the diversity and environmental relationships revealed through DNA biomonitoring can guide culturing experiments toward potential target organisms in functional ecology (Burge et al. 2018). Diatoms, used in concert with other water quality monitoring proxies, are excellent indicators of aquatic ecosystem health due to their ability to respond rapidly to change and because of their ornate silica frustules with species-specific patterns that can persist as an ecological fingerprint by providing species level identification (Smol and Stoermer 2010). These characteristics combined with rigorous diatom ecological examination have led to a wealth of knowledge about diatom-environmental species indication for rivers (Potapova and Charles 2007, Reavie and Smol 1998), wetlands (Wachnika et al. 2010, Justus et al. 2016), and lakes (Camburn and Charles 2000, Ramstack et al. 2003, Reavie and Kireta 2015) in North America. In paleolimnology, preserved diatom microfossils have been highly effective in the reconstruction of lake histories and informed science and management on a wide range of issues from recent human impact to reconstructing climate and ecology on millennial time scales (Smol and Stoermer 2010).

In the last two decades methods for DNA analysis have advanced the description of the diatom genome, providing insights into diatom metabolic function (Armburst et al. 2004, Vidoudez and Pohnert 2013) and environmental DNA biomonitoring (Zimmerman et al. 2015, Kelly et al. 2018, Vasselon et al. 2019, Wang et al. 2019, Apothêloz-Perret-Gentil et al. 2020, Epp et al. 2011, Stoof-Leichsenring et al. 2012, 2015). While the foundations of comprehensive environmental DNA bio-monitoring programs have been established in Europe (Kelly et al. 2018, Vasselon et al. 2017a, 2019), there are a smaller number of pioneering studies for North American rivers (Minerovic et al. 2019, Mora et al. 2020, Smucker et al. 2020) and none for lakes. With the assistance of updated European diatom genomic taxonomic databases (Rimet et al. 2016, 2018, Yilmaz et al. 2014), high-resolution bioinformatics (Callahan et al. 2016, Seigewald et al. 2017, Tapolczai et al. 2019), and phylogenetics (Capo et al. 2015, Shirouzu et al. 2020), here we characterize diatom assemblages, species, and within-species variability across space, time, and environmental gradients for lakes in Minnesota, USA.

Minnesota encompasses over 12,000 lakes spanning seven diverse ecoregions, with most lakes contained in three: the Western Cornbelt Plains, North Central Hardwood Forest, and the Northern Lakes and Forests (Omernik 1987). Lakes in these ecoregions span a wide range of depths, ion concentrations, and nutrient gradients (Moyle 1956). In turn, these water chemistry gradients result in different ecologies of lakes across the state (Eddy 1938, Moyle 1945, Bright 1968, Burgam 1983). The diatom taxonomy and

ecology of Minnesota lakes has been intensively studied during the last century (Edlund & Stoermer 2013). For example, Koppen (1978) found morphological strains of *Tabellaria flocculosa* (Roth) Kützing to be distributed along pH and trophic gradients. Brugam (1983) examined 105 Minnesota lakes to find that surface sediment diatom assemblages were indicative of lake type, pH and trophic gradients. In addition to these surface water quality and community variations, many lakes also differ in history owing to a variety of human impacts over the last two centuries. The use of diatoms to reconstruct historical records in Minnesota lakes is extensive (Bradbury and Megard 1972, Bradbury 1975, Bradbury and Winter 1976, Forester et al. 1987, Bradbury 1988, Dean et al. 1994, Tracey et al. 1996, Card 1997, Bradbury et al 2002, Ramstack et al. 2003, Ramstack et al. 2004, Serieyssol et al. 2009, Reavie and Edlund 2013, Ramstack-Hobbs 2016, Reavie et al. 2017); there are also diatom paleo-reconstructions on Minnesota wetlands (Brugam 1978, Brugam and Swain 1999). Card (1997) showed that historically, in Big Watab Lake, oligotrophic species like *Lindavia bodanica* (reported as *Cyclotella bodanica*) decreased in abundance concurrent with indicators of watershed clearing in the late 19th century; beginning in the 1930s the more eutrophic *Stephanodiscus minutulus* increased and was dominant for most of the 20th century. Bradbury et al. (2002) highlights the accumulation of knowledge gained through decades of diatom paleolimnology on Elk Lake, MN. In this study, diatoms were used to reconstruct sunspot periodicity/climate of the region and increased productivity coinciding with logging of the watershed. Ramstack et al. (2003, 2004) used modern lake

sediments paired with water chemistry samples to develop a weighted average model, referred to as a transfer function, for reconstructing total-phosphorus concentrations in Minnesota lakes. Heiskary and Wilson (2008) combined the efforts of Ramstack et al. (2003, 2004) with regulatory needs in order to establish nutrient standards that accounted for historical, pre-disturbance conditions. The extensive knowledge of diatom taxonomy and ecology in Minnesota is a crucial foundation for examination of novel bio-monitoring approaches such as metabarcoding in paleo-lake sediments (Domaizon et al. 2017).

One of the early hurdles to using diatom metabarcoding approaches was identifying regions of genetic variability suitable for genus and species level discrimination. Amplicons are short segments of DNA defined by primers on each side of the region. Amplicons of DNA from the ribosomal, mitochondrial, internal transcribed spacers, and carbon fixation genes have provided enough variability to inform phylogenetic understanding and taxon delimitation within the phylum Bacillariophyta (Evans et al. 2007, 2008, Alverson 2008, Hamsher et al. 2011, Zimmerman et al. 2011, Kermarrec et al. 2013). Constructing single gene phylogenetic trees, Gou et al. (2015) showed that that the small ribosomal subunit (18S) and the chloroplast gene ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (*rbc*L) are useful for genus and species level taxonomic delineation. Efforts have been made to construct and curate high quality taxonomic databases for these two amplicon regions (Zimmerman et al. 2014, Rimet et al. 2016, 2018).

Another area of recent progress is the advancement of bioinformatic approaches to identify individual taxa using taxonomy free approaches. Here a taxonomy free approach is defined as using various methods to delineate and analyze all unique DNA sequences detected. Tapolczai et al. (2019) compared ecological metric performance using taxonomy free approaches including operational taxonomic units (OTUs), exact sequence variants (ASVs), and individual sequence units (ISUs), and found that ASVs and ISUs outperformed OTUs. Taxonomic assignment can be assigned to ASVs, ISUs, and OTUs using reference databases and Bayesian classifiers (Wang et al. 2007). Although general community characteristics have been captured in diatom eDNA approaches, discrepancies in community composition appear when comparing abundances and presence/absence of taxa between light microscopic and molecular sampling (Zimmerman et al. 2015, Dulias et al. 2016, Vasselon et al. 2017c, Stoof-Leichsenring et al. 2020a). Therefore, care must be taken when incorporating molecular approaches into existing biotic indices (Pawlowski et al. 2018) or transfer functions (Domaizon et al. 2017).

With the advancement in environmental DNA approaches, diatom community structure from streams and lake sediments has been successfully tied to environmental condition gradients. Beginning in the late 2000s, studies used eDNA to characterize diatom communities (Jahn et al. 2007), and diatom molecular bio-monitoring programs have been applied to river biomonitoring in the United Kingdom (Kelly et al. 2018), France (Vasselon et a. 2019), and Scandinavia (Bailet et al. 2020).

Recently riverine studies in North America have used eDNA to characterize the diatom communities and their relations to environmental variables (Craine et al. 2017, Minerovic et al. 2019, Mora et al. 2020, Smucker et al. 2020). Smucker et al. (2020) demonstrated the ability of a taxonomy free approach using *rbc*L amplicons to indicate total nitrogen and total phosphorus conditions for a watershed in Ohio. Despite the successful implementation in stream environments, DNA biomonitoring in North American lakes has not yet been attempted. Epp et al. (2011) and Stoof-Leichsenring et al. (2012) demonstrated that diatom DNA could be successfully recovered from lake sediments and amplicon sequencing could be used to identify the dominant taxa that were also observed in light microscopy. Revealing diversity not apparent in traditional microfossil identification, Stoof-Leichsenring et al. (2015) used eDNA from a lake sediment core to identify intraspecific variability within *Staurosira* associated with tree line movement within the watershed. Rivera et al. (2018) examined the benthic diatom community in France's Lake Bourget, finding congruence in molecular and microscope diatom detections and identifying environmental factors that controlled floristic variation within the lake. Capo et al. (2017) found no significant effects of sediment core diagenesis on the diatom community composition based on eDNA in a repeated coring experiment. Recently Stoof-Leichsenring et al. (2020a) described the similarities and differences of species abundance when comparing light microscopy and molecular methods in surface lake sediments. They found some taxa such as *Aulacoseira* well represented in lake sediments while others like *Tabellaria* were

abundant in light microscopy but were not detected in the amplicon sequencing approach.

Moving forward in a region rich with lakes and limnological research and building upon the research for DNA detection in lakes and streams, here we: 1) evaluate the ability to recover diatom DNA from lake sediments, 2) describe the diversity of planktonic diatoms in these lakes using *rbc*L and 18S amplicon sequence variants, and 3) determine if lake sediment DNA reflects the conventional diatom composition, assemblage changes, and limnological change through space and time.

5.2 Methods

This study relied on subsamples from the Science Museum of Minnesota/St. Croix Watershed Research Station's (SMM/SCWRS) "*Cylindro*" project which examined 20 Minnesota Sentinel Lakes for statewide distribution of *Cylindrospermopsis* (Heathcote et al. in prep.). Wet material from recently collected surface sediments and sediment cores was sealed under nitrogen and frozen at -20°C. Subsamples from 20 modern and 30 paleo lake sediments, were transported on dry ice to the University of Minnesota for DNA extraction for eukaryotic amplicon sequencing (Table 5.1, Figure 5.1). Ecoregions examined here were defined by the Minnesota Department of Natural Resources and include the Border Lakes Subsection (referred to hereafter as Canadian Shield), the Laurentian Mixed Forest Province (referred to hereafter as Big Woods), the northern section of the Eastern Broad Leaf Forest Province, (referred to hereafter as Transition Forest), and the southern section of the Eastern Broad Leaf

Province combined with the North Central Glaciated Plains Section (referred to hereafter as the Corn Belt). Average available water quality (e.g.; Total Phosphorus, Chlorophyll-a, Secchi depth, and Carlson Trophic State Index) and watershed characteristics (e.g.; area and percentage of pasture, forest, developed, and cultivated) were obtained from the Minnesota Pollution Control Agency reports obtained through the Minnesota Department of Natural Resources Lake Finder webpage (MNDNR 2020).

For taxonomic comparisons between eDNA methods and traditional diatom enumeration by light microscopy, diatom counts were used from previous paleolimnology projects from the St. Croix Watershed Research Station on Hill and South Center Lakes. Briefly, cores were taken from the ice surface or from a boat using a Al-Zr drive rod and piston-coring system (Wright 1991). Cores were maintained in an upright position until the top 30-40 cm were sectioned; further sectioning occurred upon return to the SCWRS laboratory. Cores were sectioned in 2 cm increments to the bottom of each core. Loss on ignition was performed following Dean (1974) to determine general sediment composition (e.g. organic, inorganic, and carbonate content). Radioisotopic dating of the sediments was performed using ^{210}Pb (Appleby and Oldfield 1978, Appleby 2001, Binford 1990). Loss on ignition was used to match lithologic changes in the South Center Lake core with those in a previously dated core collected from the exact same location, with age interpolation between dated marker horizons. Diatom microfossils were separated from lake sediments using a water bath for 4 hours at 80°C (Ramstack et al. 2008), where “cleaned” materials were then dried onto 22x22 mm No. 1

coverglasses and mounted on slides using Zrax mountant. For most samples, 400 valves were counted and identified to the lowest taxonomic level (Reavie and Smol 1998, Camburn and Charles 2000, Fallu et al. 2000, Reavie and Kireta 2015, Spaulding et al. 2020).

5.2.1 *DNA extraction and sequencing*

DNA extraction was performed from 200-250 mg of frozen lake sediments by the University of Minnesota Genomics Center (UMNGC, Minneapolis, Minnesota, USA) using a DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's instructions starting with Solution CD1 and final elution of DNA in 66 *µ*L. The UMGC verified and quantified DNA isolation using NanoDrop UV spectroscopy (Simbolo et al. 2013) and PicoGreen fluorometry (Ahn et al. 1996, Enger 1996) (Table 5.1). Six control blanks were also randomly included during the extraction process, all resulting in no detection of DNA. The extracted DNA was then transferred within the UMNGC for amplification. Subsamples of extracted DNA were amplified for two target amplicons, specifically targeted for retrieving taxonomic information relevant to diatoms, the 18S V4 region (Zimmerman et al. 2011; 18S V4 Region D512 Forward 5' ATTCCAGCTCCAATAGCG 3', D978 Reverse 5' GACTACGATGGTATCTAATC 3') and the chloroplast marker *rbc*L (Kelly et al. 2018; *rbc*L-646 Forward: 5' ATGCGTTGGAGAGARCGTTTC 3', *rbc*L-998 Reverse 5'GATCACCTTCTAATTTACCWACAACTG 3'). The target regions are 466 bp and 352 bp for 18S V4 and *rbc*L, respectively. Amplification products were checked for fragment size using electrophoresis (Supplemental Figure 5.1).

Amplified products were multiplexed using dual indexing (Gohl et al. 2016) and sequenced on the UMNGC's Illumina MiniSeq next generation sequencing platform, yielding 300 bp forward and reverse reads. Reads were demultiplexed by the UMNGC and downloaded for downstream analyses.

5.2.2 *Extraction and QAQC*

Paired-end Illumina reads were downloaded from the UMNGC as FASTQ files and imported into R (R Core Team 2014) using R Studio (RStudio Team 2020). *Cutadapt* was used to remove forward and reverse primers as well as their inverted complements (Martin 2011). Reads were then analyzed following the *dada2* pipeline (Callahan et al. 2016, Callahan et al. 2017). Upon examination of the Phred quality scores and error rates for each sample, the reads were filtered and trimmed to 250bp forward and 230bp reverse for both *rbc*L and 18S with a maximum error rate of two forward and five reverse. Identical reads were dereplicated, and using the learned error rates, unique forward and reverse reads were then determined. Unique reads were then matched and merged together with their forward and reverse complements. Merged reads were then filtered for chimeric reads using the consensus method resulting in taxonomic units as defined by unique sequences, referred to as amplicon sequence variants (ASV).

5.2.3 *Taxonomic assignment*

Taxonomy was applied to sequence data using the Naïve Bayesian Classifier algorithm within the '*assignTaxonomy'* function from the *dada2* R package (Wang et al. 2007, Callahan 2016). For both amplicon datasets,

taxonomic matches with bootstrap values of 85% or greater provided a balance of informative taxonomy and maintaining ASV richness. The 18S amplicon ASVs were paired with taxonomy using the Silva database version 138 (released December 16, 2019) (Yilmaz et al. 2014). The Diat.barcode R package was used to import *rbc*L diatom reference sequences (Rimet et al. 2018). Inconsistencies are present for phylum, class, and order taxonomic placements and nomenclature between the Diat.barcode and Silva databases, as well as when compared with curated diatom databases for taxonomic consistency (Guiry et al. 2014, Spaulding et al. 2020). Rather than reformatting or altering the genomic databases prior to analyses, we worked with our taxonomic understanding to apply taxonomic inferences (Table 5.2), with the understanding that relationship definitions within Bacillariophyta are still evolving (Nakov et al. 2015, 2018, Ruck et al. 2016).

5.2.4 *Community Statistics*

Alpha diversity and Simpson's diversity were calculated on ASVs to identify differences in communities in the R package '*Phyloseq'* (McMurdie and Holmes 2013). Counts of DNA copies within each sample were summed for all Bacillariophyta identified at the genus level and then the read copies for each ASV occurring within that sample were divided by the total read sum to determine relative abundance. Heat maps were generated for Coscinodiscophyceae, Mediophyceae, and Fragilariales/Tabellariales to describe temporal and geographical ASV distribution. To further describe the distribution of ASVs, relative reads were transformed using the Hellinger method (Legendre

and Gallagher 2001) and ordinated with non-metric multidimensional scaling using Bray-Curtis distance measures in the *Vegan* R package (Oksanen et al. 2013). The order of appearance of ASVs in the heatmaps is determined using the Bray-Curtis distances (Rajaram and Onono 2010). Within the sediment cores, Hellinger-transformed relative ASVs constrained cluster analysis (CONISS) to characterize differences and similarities between samples (Juggins and Juggins 2019), where significant clusters were determined using the broken stick model (MacArthur 1957). Phytoplankton ASVs from 18S and *rbc*L were analyzed against lake total phosphorus concentration for potential indicator value using Threshold Indicator Taxa Analysis in the R package *TITAN2* (Baker and King 2010). The ASV table was pruned to taxa with three or more occurrences. Parameters for conducting TITAN included 1,000 permutations and 1,000 bootstrap runs; however, given a low sample size (n=17), model parameters were relaxed to 80% confidence intervals and 80% purity.

5.3 Results

5.3.1 *Extraction and QAQC*

A range of 67.7 ng to 5,308 ng of DNA was recovered from the bulk lake-sediment extractions (Figure 5.2); raw DNA reads from Illumina sequencing varied from 3 to 61,853 per sample (Table 5.1). The UMNGC quality control blanks recorded up to 877 reads. Within the range of UMNGC quality controls and low quality Phred scores, surface samples from Cedar Lake, Elk Lake, and Greenwood Lake, and two sediment-core samples from each of Hill Lake (24-26 cm and 34-36 cm) and South Center Lake (96-98 cm and 120-122 cm) were

removed from further analysis. The South Center Lake *rbc*L sediment sample interval 24-26 cm also had low reads, a low Phred score, and failed quality control and was also discarded. This resulted in 43 samples for 18S (1,508,748 raw reads) and 42 samples for *rbc*L (1,266,089 raw reads) passing sequencing quality control. On average 76% of reads were retained after merging paired ends and removing chimeric sequences. Filtered non-chimeric *rbc*L reads ranged from 2,121 to 37,598 with an average of 22,941 reads per sample (Supplemental Table 5.1) and filtered non-chimeric 18S reads ranged from 3,222 to 54,398 with an average of 26,523 reads per sample (Supplemental Table 5.2). These reads represented a range of 75 to 592 18S ASVs and 63 to 342 *rbc*L ASVs identified for each site, with a total of 4454 and 2251 fully overlapping ASVs for 18S and *rbc*L, respectively (Figure 5.2).

The taxonomic richness of *rbc*L ASVs was on average 144, ranging from 60 to 341 taxa (Figure 5.3). Simpson's diversity was on average 0.95, ranging from 0.77 to 0.99, Artichoke (0.81) and Shaokatan (0.77) lakes appeared to be more dominated by a few taxa compared to the other surface sediments and sediment cores. The taxonomic richness of 18S ASVs was on average 245 ranging from 60 to 452 taxa (Figure 5.4). Simpson's diversity was on average 0.93 ranging from 0.61 to 0.99. South Center Lake sediment core samples from 78 cm, 86 cm, and 90 cm depth had lower Simpson's diversity.

5.3.2 *Taxonomic assignments*

The *rbc*L amplicon classified 1,943 out of 2,251 ASVs as Bacillariophyta at 85% bootstrapping confidence, and for the class taxonomic rank, 626 ASVs were

classified as either “Bacillariophyceae” (339), “Coscinodiscophyceae” (53), “Fragilariophyceae” (156), and “Mediophyceae” (62) with 1,625 ASVs unclassified (Supplemental Table 4.3, Figure 4.5). The *rbc*L “Bacillariophyta” relative reads were dominant (>50%) in 27 samples, moderate (50%-25%) in 8 samples, and was a minor (<25%) portion of the recovered *rbc*L DNA in 6 samples. At the genus taxonomic rank, 486 (21.6%) of the ASVs were classified among 39 genera, and 214 ASVs were classified among 84 species (9.5%, Table 5.3, Supplemental Table 5.3).

The Silva 138 database classified 311 out of 4454 18S ASVs as Bacillariophyta, reported in the database as “Diatomea”, at 85% bootstrapping confidence (Figure 5.6). In absence of diatom class level classifications, ASVs were classified at the order level as “Bacillariophyceae_or” (100), “Coscinodiscophytina_or” (24), “Fragilariales” (62), and “Mediophyceae_or” (54) with 71 unclassified ASVs (Figure 5.6). At the genus rank 185 (60%) of the 18S ASVs were classified within 20 genera and ASVs could not be classified as species at this time (Figure 5.6, Table 5.3, Supplemental table 5.4).

5.3.3 *rbc*L *ASV Distributions and Environmental Signals*

Sample sites or lakes distributed in non-metric multidimensional scaling (NMDS) space by *rbc*L diatom ASV abundance were generally clustered within their respective ecoregions (Stress = 0.14, 2D, $r^2 = 0.9$, Figure 5.7). Samples from the Canadian Shield ecoregion and The Big Woods ecoregion lakes were positive on NMDS axis 2. Sites in the Northern Lakes and Forests ecoregion plotted generally positive on NMDS axis 1 and negative on NMDS axis 2. The

samples representing lakes in the Corn Belt ecoregion were negative on NMDS axis 1 and generally negative on NMDS axis 2. Heatmaps revealed that *rbc*L ASVs within Coscinodiscophyceae, Fragilariophyceae, and Mediophyceae varied in frequency and distribution (Figure 5.8). Genera such as *Aulacoseira, Fragilaria, Staurosira, and Stephanodiscus* were composed of numerous ASVs distributed across many lakes and ecoregions, whereas the genera *Acanthoceras, Asterionella, Cyclostephanos, Cyclotella, Discostella, Pantocsekiella, Lindavia, Melosira, Tabellaria,* and *Urosolenia* were often confined to only a few ASVs and had limited occurrences. Temporal and geographic variability of *rbc*L ASVs occurred within and between genera and species. For example, *Cyclostephanos tholiformis* was only detected in Peltier Lake, yet the species occurrence there was composed of 5 ASVs (ASV: 587, 673, 646, 716, and 1,206). In contrast the closely related *Cyclostephanos invisitatus* clade, composed of 3 ASVs (408, 695, and 1,256) was detected in Belle, Hill, Peltier, and South Center lakes. *Aulacoseria* contained both cosmopolitan and historical sequence variants, with some ASVs (1, 2, 3, 5, 7, 9, 11, 13) occurring throughout Hill and South Center lakes' histories as well as in most lakes examined. There are several *Aulacoseira rbc*L ASVs only found in the sediments of Hill and South Center Lakes, ASVs 1848, 1819, 397, 1067, 972, 447, 671, 559, and 190, 179, 38, 26, respectively.

Environmental variables were found to be significantly correlated with the *rbc*L ASV ordination. Secchi depth, total suspended solids, total phosphorus, chlorophyll-a, and cultivated land-use cover were significantly correlated with the

negative NMDS axes, and lake depth was correlated with the positive NMDS axes (Figure 5.9). Six *rbc*L ASVs representing *Aulacoseira granulata* (4, 6, 10, 88), *Staurosirella martyi* (80), and *Cyclotella sp*. (191) were found by TITAN to be indicators of high total phosphorus, and five *rbc*L ASVs of *Staurosira sp.* were found to be indicators of low total phosphorus (Figure 5.10).

5.3.3 *18S ASV Distributions and Environmental Signals*

Nonmetric Multi-Dimensional Scaling characterized the diatom 18S ASVs in a 2-dimensional solution (Stress= 0.09, linear fit r^2 = 0.967, Figure 5.11). Lakes from the Canadian Shield ecoregion were positive in the NMDS axes 1 and 2, Northern Lakes and Forests samples were plotted near the origin of NMDS axis 2, with Portage and Red Sand lakes near the maximum of NMDS axis 1. Many of the WCBP lakes were clustered near the origin with Artichoke and Shaokatan near the minimum of NMDS axis 2. The Big Woods lakes were also distributed near the origin with Carlos and Pearl lakes occurring negative on NMDS axis 1. *Aulacoseira* and *Staurosira* composed the most identified 18S ASVs at the genus level occurring in lakes from all ecoregions, with *Aulacoseira* distributed along NMDS axis 2 and *Staurosira* mostly positive on NMDS axis 1 (Figure 5.11). *Cyclotella* and *Fragilaria* were common in Big Woods lakes, with *Thalassiosira* occurring in Corn Belt lakes and *Stephanodiscus* in Corn Belt and Big Woods Lakes. The 39 18S ASVs not classified at the genus level were varied in their distribution.

Many “Coscinodiscophytina_or” ASVs were infrequent in their occurrence and distribution; exceptions were ASVs 2 and 8 which were detected in most

lakes and through most of the sediment cores (Figure 5.12). "Coscinodiscophytina_or" appeared to generally be restricted to Trout and White Iron Lakes, along with the most ancestral ASVs 656 and 4389. Within Fragilariales, *Fragilaria* 18S ASVs were Carrie, Hill, Pearl, and St. James lakes, with many *Staurosira* occurring widespread (ASVs; 72, 102, 134, 153, 201, 454, 717) and some restricted in distribution (ASVs; 836, 841, 395, 1160, 1752, 1304, 2020, 3713). Within "Mediophyceae_or", many ASVs were restricted in their occurrence to one or just a few samples. An exception was ASVs 3, 36, 132, 140, which were distributed through time and across several lakes.

Four environmental variables were significant with the 18S ASV NMDS (Figure 5.13). Cultivated land-use and total phosphorus were significant to the negative end of NMDS axis 2, whereas forested land-use and lake depth were significant to the positive end of NMDS axis 2. The 18S ASVs 8 (*Aulacoseira*) and 275 (an unknown "Coscinodiscophytina_cl") were identified by TITAN as indicators of high phosphorus; no 18S ASVs were identified to be significant indicators of low phosphorus (Figure 5.10).

5.3.4 *Diatom LM and DNA Reconstructions*

Both the 2014 and the 2018 sediment cores from Hill Lake recovered sediments back to 1880. In brief, the diatom history based on microscopy has been marked by a significant change in the mid 20th century, where a modern community composed of *Asterionella formosa*, *Aulacoseira ambigua*, *Fragilaria*, *Stephanodiscus niagarae,* and *S. hantzschii* replaced the historical community composed of *Aulacoseira granulata, Stephanodiscus minutulus,* and *Staurosira*

(Figure 4.14). *Cyclostephanos invisitatus* and *Staurosirella martyi* occur sporadically throughout the sediment record. The *rbc*L diatom reconstruction was also distinguished by a significant break in the mid-20th century (Figure 5.15). The *rbc*L reads, as relative abundance, recorded a modern community of *Aulacoseira ambigua*, *Stephanodiscus minutulus*, *S. yellowstonensis*, *Staurosira*, *Pseudostaurosira brevistriata*, and *Staurosirella martyi,* which replaced a historical community composed of *Aulacoseira ambigua, A. granulata, Cyclostephanos invisitatus, Fragilaria,* and *S. hantzschii*. *Asterionella formosa* and *Stephanodiscus minutulus* occur variably throughout the sediment record. The significant break in the Hill Lake 18S diatom reconstruction occurs in the mid-20th century (Figure 5.16). The post-1950s community is composed of fewer *Aulacoseira* and *Fragilaria*, and increased Mediophyceae and *Staurosira* compared to the historical community. In Hill Lake, *Lindavia bodanica*, *Cyclostephanos tholiformis*, *Cyclotella*, *Stephanodiscus medius*, and *Stephanodiscus parvus* were reported with light microscopy, however they were not readily resolved using amplicon sequencing.

In the 2019 South Center Lake core, light microscope diatom analysis revealed a significant change around 1980, with the modern community composed of *Aulacoseira ambigua*, *A. granulata*, *Cyclotella, Fragilaria*, *Stephanodiscus minutulus, S. niagarae*, and *Pseudostaurosira brevistriata* (Figure 5.17). While *Stephanodiscus hantzschii* does occur in the modern community, it rapidly disappears after the earliest sample within this cluster group. The historic community is described as *Aulacoseira ambigua*, *Staurosira*,

and *Stephanodiscus hantzschii* with lesser occurrences of *A. granulata, Fragilaria, and Staurosira*. *Staurosirella martyii* was found at the very top of the core, and *Urosolenia* occurred sporadically throughout the core. The diatom *rbc*L sediment record in South Center Lake captured a significant change in the mid 20th century (Figure 5.18). The modern community was composed of *Aulacoseira ambigua, A. granulata*, *Cyclotella*, *Fragilaria, Stephanodiscus hantzschii*, *S. minutulus, S. yellowstonensis, Staurosira, Pseudostaurosira brevistriata,* and *Staurosirella martyi.* The historical community was composed of *lesser abundances of Aulacoseira ambigua*, *A. granulata*, and *Staurosirella martyi*, in addition to *Fragilaria, Stephanodiscus minutulus*, and *Staurosira venter. Urosolenia eriensis* occurred occasionally throughout the core. The 18S ASVs recorded one significant break around 1930, with trace or non-detections of diatoms during the mid-20th century (Figure 5.19). The modern sediments record a community composed of *Aulacoseira, Cyclotella*, Mediophyceae*,* and *Staurosira*. This community composition was also recorded in the most ancient sediments observed from South Center Lake, although abundances were very low. *Asterionella formosa, Cyclostephanos tholiformis, Discostella, Stephanodsicus alpinus*, and *S. parvus* were common diatoms reported with light microscopy, although they were not readily identified or detected using amplicon sequencing.

5.4 Discussion

We undertook the first comprehensive study for the potential application of environmental DNA for assessing ecological change in Minnesota and North

American lakes. Useable diatom DNA was recovered from most lake surface sediment and paleolimnological samples. The diatom community composition detected using molecular techniques was consistent with ecoregional lake classifications and diatom community environmental patterns detected using microscopy. In Hill Lake, diatom microfossils and eDNA (*rbc*L and 18S) detected assemblage changes consistent with watershed development in the mid 20th century. Preliminary investigation on this limited dataset identified 13 ASVs which were significant indicators of nutrient differences.

There were not significant trends between bulk sediment DNA recovery and the overall quality of the reads, however extractions which yielded less than 2,000 ng of DNA were prone to contain poor quality reads. Overall, the sequence reads recovered here were within the range from other recent studies (Tapolzacai et al. 2019, Stoof-Leichsenring et al. 2020a). In contrast to recent stream survey data (Apothéloz-Perret-Gebtil et al. 2020), we observed only a slightly higher number of 18S reads when compared to *rbc*L reads, although on average, 18S produced 101 more ASVs per site compared to *rbc*L. From samples containing high quality reads, there appeared to be no differences in community diversity measures between historical and modern lake sediment samples. However, there was lower community diversity in 18S amplicon sequence variants (ASV) for the three oldest South Center Lake sediment core samples, and in *rbc*L surface communities of Artichoke and Shaokatan Lakes. While species taxonomic assignments are currently lacking for our 18S observations, it is possible the diversity losses observed could be attributed in

South Center lake to a dominance of *Aulacoseira ambigua* as reflected in the corresponding *rbc*L and diatom microfossil record. Lower *rbc*L diversity of Artichoke and Shaokatan Lakes, both subjected to nutrient pollution and harmful algal blooms, were dominated by the nutrient tolerant *A. granulata*, which may be attributed to environmental disturbance and/or competition with cyanobacteria. This study leveraged existing paleo-sediment achieves and a majority of samples produced ample quantities of high-quality DNA. Future Minnesota studies should emphasize sterile techniques during sediment subsampling and extraction (Epp et al. 2019) as well as understand DNA recovery along gradients of sediment geochemical composition (Capo et al. 2020) and sedimentation rates (Capo et al. 2017).

Both the *rbc*L and the 18S ASVs were useful in capturing some of the diatom genetic diversity in Minnesota lakes. However, based on the databases examined here, *rbc*L was able to identify 1632 more diatom ASVs. Differences noted between 18S and *rbc*L could be a reflection of amplicon molecular diversity (Alverson 2008, Guo et al. 2015), but may also be due to bias from database selection. We used a well curated diatom *rbc*L database (Rimet et al. 2018) as compared to the 18S database curated for a larger group of organisms including Bacteria, Archaea, and Eukarya (Yilmaz et al. 2014). Relaxing database matching from 100% to 85% bootstrapping allowed for twice the amount of taxonomic information. While this was a useful approach working in a new geographic region, as taxonomic databases are developed for diatoms in Minnesota, we encourage increasing the bootstrap confidence levels to 100%.

Stephanodiscus yellowstonensis and *S. suzuki* are considered endemic to their type localities and have not been reported in Minnesota. *Stephanodiscus niagarae*, common in Minnesota (Ramstack et al. 2003, 2004), is a close morphological relative to *S. yellowstonensis* (Theriot et al. 2006) but is not represented in the *rbc*L database. While several taxa observed using light microscopy in Hill and South Center Lakes highlight taxonomic gaps with genomic databases, the genomic record of *Tabellaria* requires special examination. As observed by Stoof-Leichsenring et al. (2020a), *Tabellaria* frustules are persistent in the Hill Lake sediment record whereas the *rbc*L record of *Tabellaria* rapidly diminishes down core. Future improvements to ASV taxonomy could be made using phylogenetic associations (Keck et al. 2016, Stoof-Leichsenring et al. 2020b).

Lakes in Minnesota are ecologically differentiated across the ecoregions of Minnesota. Heiskary et al. (2008) and Ramstack et al. (2002, 2003) showed that the diatom communities were similarly distributed by ecoregion. From a small number of lakes (n=17) we note a similar geographic delineation characterized by both the 18S and *rbc*L diatom communities. While environmental factors such as lake depth and total phosphorus were significant with diatom genomic communities, other important known drivers of diatom distributions in Minnesota Lakes were unfortunately not examined here (e.g. pH, alkalinity, and specific conductance) and should be assessed in future studies. The identification of *Aulacoseira granulata* and *Staurosirella martyi* as high total phosphorus indicator species based on DNA is consistent with their community

association with eutrophic waters (Witkowski et al. 1995, Kirilova e al. 2010). The utility of diatom genera as bioindicators using eDNA tends to be coarse such that species-level taxonomy for high TP *Cyclotella* ASVs and low TP *Staurosira* ASVs is warranted. Here we only identified a fraction of indicator species compared to Smucker et al. (2020), highlighting the need for a study with a larger sample size to more fully understand diatom genomic biomonitoring within the diversity of Minnesota lakes (Moyle 1956).

Historical diatom responses revealed a consistent timing of ecological change in Hill Lake between microscope and molecular methods. However, the South Center Lake record illustrated varied responses between the methods, especially the notable absence of ASVs during the mid-20th century. Using diatom microfossils and to some extent *rbc*L ASVs, Hill Lake appears to slightly improve in water quality over the 20th century. The assemblage change suggests a shift from nutrient-tolerant *A. granulata* and *S. minutulus* to a more mesotrophic community of *A. ambigua* and *Tabellaria* (Camburn and Charles 2000). The diatom microfossil stratigraphy from South Center Lake indicated a late 20th century shift toward greater productivity, corresponding to increases in sediment phosphorus concentrations and a shift toward more nutrient efficient *Daphnia* (Frisch et al. 2017). Disparities between microfossil enumeration and metabarcoding are common (Zimmerman et al. 2015, Mora et al. 2019); suggesting that calibration factors be developed to account for cytoplasmic difference in DNA concentrations among species (Godhe et al. 2008, Vasselon et al. 2017c).

5.5 Conclusion

Diatom phytoplankton community assemblages can be recovered from lake sediments using amplicon sequencing in Minnesota lakes. Furthermore, the metabarcoding approach captured assemblage differences along environmental gradients across many lake types. Diatom microfossils records indicate improvements to water quality (lower nutrient levels) in the recent history of Hill Lake which parallel the *Daphnia* and sediment phosphorus response to nutrient enrichment in South Center Lake. Overall lake sediment bulk DNA proved useful by describing trends similar to the diatom microfossil assemblage and my analysis revealed areas to improve the methodology including: 1) the development of taxonomic libraries, 2) establishing calibration factors for important taxa, and 3) understanding the preservation of DNA in lake sediments.

Tables

Table 2.1. Sediment core sampling and analysis parameters including the identification number (ID), year recovered, latitude and longitude, lake depth, length of sediment core recovered, and downstream analysis conducted on each core.

Core ID	Recovery Year	Lat. °N	Long. °W	Depth (m)	Length Recovered (m)	Analyses
LC1	2016	47.99023	-95.15025	7.47	0.81	P-frac., diat.
LC2	2016	47.96323	-94.87279	7.97	0.81	P-frac., diat.
LC3B	2016	48.00795	-95.03203	7.72	0.69	P-frac., diat., pigs.
UC1	2016	48.10329	-94.94996	4.66	0.84	P-frac., diat.
UC1B	2018	48.10331	-94.94995	4.4	1.01	pigs.
UC2A	2016	48.12668	-94.75829	4.63	0.81	-
UC3	2016	48.14822	-94.61871	4.2	0.80	-
UC4	2018	48.15018	-94.85711	4.2	1.02	P-frac., diat.

Table 2.2 The mean and percentiles for Lower Red Lake; monitored total phosphorus (TP), modern diatom-inferred TP (DI-TP), historical DI-TP, chlorophyll-a, phaeophytin corrected chlorophyll-a.

Lower Red Lake	Mean	25th Percentile	75th Percentile	85th Percentile	90th Percentile
Monitored TP (µg/L)	37.2	28	45	48	52
Modern DI-TP (µg/L)	43.9				
Historical DI-TP (µg/L)	43.5				
Chlorophyll-a (µg/L)	10.4	7	14	16	17.3
Corr. Chlorophyll-a (µg/L)	11.7	7	16	18.64	20.84
		10th Percentile	**15th Percentile**	**25th Percentile**	**75th Percentile**
Secchi Depth (m)	1.1	0.76	0.76	0.91	1.22

Table 2.3 The mean and percentiles for Upper Red Lake; monitored total phosphorus (TP), modern diatom-inferred TP (DI-TP), historical DI-TP, chlorophyll-a, phaeophytin corrected chlorophyll-a.

Upper Red Lake	Mean	25th Percentile	75th Percentile	85th Percentile	90th Percentile
Monitored TP (*µ*g/L)	45.9	35	54	60	63.9
Modern DI-TP (*µ*g/L)	37.7				
Historical DI-TP (*µ*g/L)	38.7				
Chlorophyll-a (*µ*g/L)	16.3	12	20	23	25
Corr. Chlorophyll-a (*µ*g/L)	12.89	8	16.58	19	20.7
		10th Percentile	15th Percentile	25th Percentile	75th Percentile
Secchi Depth (m)	0.66	0.15	0.46	0.61	0.91

Table 3.1. Distribution of dormant propagules among freshwater organisms including mode of production, viability, trigger for dormancy, conditions for resuscitation of propagules, and paleo-indicator value. References in parentheses.

Taxonomic group	Dormant Propagule	Life History	Viability (years)	Dormancy Trigger	Resuscitation	Paleo-Indicator Value
Cladocera	Ephippium (1)	Sexual, parthenogenesis (1)	600 (2)	Resource limitation, seasonality, crowding, predation (1)	Temperature, photoperiod, dissolved oxygen (1)	Climate (5), lake level (6), trophic state (7), acidification (8), species invasion (9)
Copepoda	Diapausing eggs (1)	Sexual (1)	300 (3)			Lake level (10), acidification (11)
Rotifera	Diapausing eggs (1)	Sexual, parthenogenesis (1)	40 (4)			Eutrophication (12)
Ostracoda	Diapausing eggs (13)	Sexual (13)		Temperature, photoperiod (13)	Favorable growth conditions (14)	Salinity, precipitation, temperature (15)
Porifera	Gemmule (16)	Sexual (16)	25 (17)	Osmonic pressure (18)	Temperature (18)	Alkalinity (19), salinity (20)
Cyanobacteria	Akinete (21)	Vegetative (21)	64 (22)	Temperature (21)	Temperature, photoperiod (22)	Eutrophication, temperature (23)
Dinoflagellates	Resting spore (24)	Sexual (24)	90 (25)	End of growing conditions (26)	Photoperiod (27)	Land-use changes (28)
Diatoms	Resting cell (29)	Vegetative (29)	100 (30)	Temperature, nutrients (31, 34)	Temperature, photoperiod (31, 35)	Acidification (36), salinity (37), eutrophication (38),
Diatoms	Resting spore (29)	Vegetative (29)	1 (34)	Nutrients (35)	Nutrients (35)	climate change (41), species invasion (42)

REFERENCES: (1) Gyllstöm & Hansson 2004; (2) Frisch et al. 2014; (3) Hairston et al. 1995; (4) Marcus et al. 1994; (5) Smol et al. 2005; (6) Alhonen 1994; (7) Bos 2000; (8) Nilssen & Sandoy 1990; (9) Keilty 1988; (10) Borromei et al. 2010; (11); (12) Finlay et al. 1998; (13) Hairston et al. 1990; (14) Mclay 1978; (15) Xia et al. 1997; (16) Rasmont 1954; (17) Harrison 1974; (18) Simpson & Fell 1974; (19) Harrison et al. 1979; (20) Cumming et al. 1993; (21) Miller & Lang 1968; (22) Livingstone & Jaworski 1980; (23) Li et al. 1997; (24) Rengefors et al. 2004; (25) Kling 1998; (26) Dale 1983; (27) Lundholm et al. 2011; (28) Heiskansen 1993; (29) Anderson et al. 1987; (30) McCarthy and Kreuger 2013; (31) McQuoid and Hobson 1996; (32) Härnström et al. 2011; (33) Lund 1954; (34) Nipkow 1950; (35) Sicko-Goad et al. 1986; (36) Gairrison 1970; (37) Jewson et al. 2008; (38) Camburn and Charles 2000; (39) Fritz et al. 1991; (40) Stoermer et al. 1996; (41) Boeff et al. 2016; (42) Edlund et al. 2000

Table 4.1. Sample size, minimum, maximum, and average of paleolimnological proxies measured in the Cedar Bog Lake sediments. Variables include time, dry mass accumulation rate (DMAR), the percentage and flux for organic, $CaCO_3$, inorganic, and biogenic silica (BSi) sediment components, the count and flux of diatom frustules, and the reads and relative fluxes for 23S DNA (total and diatoms).

Variable	Units	n	Min.	Max.	Avg.
Time	Julian year (C.E.)	61	1704	2015	-
DMAR	g cm^{-2} yr^{-1}	51	0.007	0.028	0.016
Organic	Percent (%)	51	53	80	67
$CaCO_3$		51	0	5	3
Inorganic		51	17	43	29
BSi-O_2		13	1	6	4
Total	23S reads	61	3220	11445	6363
Diatom DNA		61	0	2439	483
Diatom Count	# of valves	13	0	400	-
Organic	Flux (g cm^{-2} yr^{-1})	51	0.5	1.6	1.0
$CaCO_3$		51	0.0	206.0	38.0
Inorganic		51	15.4	109.8	52.2
BSi-O_2		13	1.1	14.0	7.2
Diatom Count		9	47	1438	651
Total	DNA flux (# reads*DMAR)	61	45	188	101
Diatom DNA		61	0	45	10

Table 4.2. The exact sequence variant (ESV) identification number and taxonomic assignments for common diatom 23S ESVs which met analysis criteria (≥ 3% abundance and ≥ 3 samples) in the Cedar Bog Lake sediments.

ESV ID	Phylum	Family	Genus	Species
034468	Bacillariophyta	Fragilariaceae	Asterionella	*A. formosa*
061066	Bacillariophyta	Melosiraceae	*Melosira*	*M. tropica*
000923	Bacillariophyta	NA	NA	Bacillariophyceae sp. 1 AS-2014
065006	Bacillariophyta	NA	NA	Bacillariophyceae sp. 3 AS-2014
031370	Bacillariophyta	NA	NA	NA
059731	Bacillariophyta	NA	NA	NA
037041	Bacillariophyta	NA	NA	NA
031623	Bacillariophyta	NA	NA	NA
061875	Bacillariophyta	NA	NA	NA
065127	Bacillariophyta	NA	NA	NA
032167	Bacillariophyta	NA	NA	NA
031090	Bacillariophyta	NA	NA	NA
062081	Bacillariophyta	NA	NA	NA
032448	Bacillariophyta	NA	NA	NA
001771	Bacillariophyta	NA	NA	NA
031389	Bacillariophyta	Naviculaceae	NA	NA
006396	Bacillariophyta	Naviculaceae	*Navicula*	N. sp. CCMP2746
031283	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
031852	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
031766	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
059585	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
031134	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
065072	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
065275	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
065360	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
065623	Bacillariophyta	Sellaphoraceae	*Sellaphora*	NA
009072	Bacillariophyta	Sellaphoraceae	*Sellaphora*	*S. pupula*
062324	Bacillariophyta	Sellaphoraceae	*Sellaphora*	*S. pupula*
063073	Bacillariophyta	Sellaphoraceae	*Sellaphora*	*S. pupula*
000050	Bacillariophyta	Thalassiosiraceae	*Thalassiosira*	NA
000009	Bacillariophyta	Thalassiosiraceae	*Thalassiosira*	*T. rotula*

Table 4.3. The voucher flora taxon code, genus, and species for diatom taxa identified during light microscopy which met analysis criteria (≥ 3% abundance and ≥ 3 samples) in the Cedar Bog Lake sediments.

Taxon Code	Genus	Species
AMA01	*Amphora*	*Amphora ovalis*
AUL01	*Aulacoseira*	*Aulacoseira sp.* 1
AUL03	*Aulacoseira*	*Aulacoseira ambigua*
COC01	*Cocconeis*	*Cocconeis placentula*
CRA01	*Craticula*	*Craticula cuspidata*
CYM01	*Cymbella*	*Cymbopleura inaequalis*
EPI01	*Epithemia*	*Epithemia adnata*
EUN01	*Eunotia*	*Eunotia formica*
EUN02	*Eunotia*	*Eunotia naegelii*
FRA01	*Fragilaria*	*Fragilaria mesolepta*
FRA02	*Fragilaria*	*Fragilariforma virescens*
GOM02	*Gomphonema*	*Gomphonema sphaerophorum*
GOM04	*Gomphonema*	*Gomphonema sp.* 4
GOM06	*Gomphonema*	*Gomphonema truncatum*
GOM09	*Gomphonema*	*Gomphonema sp. 9*
GOM10	*Gomphonema*	*Gomphonema sp.* 10
NEI01	*Neidium*	*Neidium sp.* 1
NEI02	*Neidium*	*Neidium iridis*
NIT01	*Nitzschia*	*Nitzschia amphibia*
PIN04	*Pinnularia*	*Pinnularia sp.* 4
SEL01	*Sellaphora*	*Sellaphora laevissima*
SEL02	*Sellaphora*	*Sellaphora sp.* 2
SEL03	*Sellaphora*	*Sellaphora pupula*
STA01	*Staurosira*	*Staurosira venter*
SYN01	*Synedra*	*Ulnaria delicatissima*
ULN01	*Ulnaria*	*Ulnaria sp.*1
ULN02	*Ulnaria*	*Ulnaria capitata*
ULN03	*Ulnaria*	*Ulnaria ulna*
ULN04	*Ulnaria*	*Ulnaria sp.*4

Supplemental Table 4.1. The genomic sample identification code, sediment depth interval (cm), and ^{210}Pb year estimate (C.E.) for the 2016 Cedar Bog Lake sediment core (Ch4.SupplementalTable1.xlsx).

Table 5.1. The sample code, lake name, latitude, longitude, sample depth interval (cm), total phosphorus (TP μg L^{-1}), and mass of DNA (ng) of 50 samples used for bulk lake sediment DNA extractions (n=50).

Sample Code	Lake	Lat.	Long.	Depth interval (cm)	TP (μg/L)	DNA (ng)
AR002	Artichoke	45.35	-96.14	0-2	84	985
BE002	Belle	44.98	-94.43	0-2	58	5308
CA002	Carrie	45.08	-94.79	0-2	46	1066
CE002	Cedar	44.96	-93.32	0-2	-	354
CR002	Carlos	45.96	-95.37	0-2	41	3735
EL002	Elk	45.87	-95.80	0-2	-	826
GW002	Greenwood	47.52	-91.63	0-2	-	1168
HI002	Hill	47.01	-93.60	0-2	47	2132
HI004	Hill	-	-	2-4	-	4405
HI006	Hill	-	-	4-6	-	2938
HI008	Hill	-	-	6-8	-	3292
HI010	Hill	-	-	8-10	-	3500
HI012	Hill	-	-	10-12	-	3002
HI014	Hill	-	-	12-14	-	2449
HI016	Hill	-	-	14-16	-	1286
HI018	Hill	-	-	16-18	-	1037
HI022	Hill	-	-	20-22	-	827
HI024	Hill	-	-	22-24	-	1055
HI026	Hill	-	-	24-26	-	473
HI030	Hill	-	-	28-30	-	1112
HI032	Hill	-	-	30-32	-	732
HI036	Hill	-	-	34-36	-	594
HI038	Hill	-	-	36-38	-	258
MA002	Madison	44.19	-93.81	0-2	80	2087
PE002	Peltier	45.18	-93.06	0-2	79	754
PO002	Portage	46.97	-95.12	0-2	56	3081
PR002	Pearl	45.40	-94.31	0-2	54	4302
RS002	Red Sand	46.38	-94.28	0-2	49	4396
SC002	South Center	45.38	-92.83	0-2	54	669
SC006	South Center	-	-	4-6	-	862
SC010	South Center	-	-	8-10	-	1819
SC018	South Center	-	-	16-18	-	1026

SC026	South Center	-	-	24-26	-	562
SC038	South Center	-	-	36-38	-	747
SC046	South Center	-	-	44-46	-	497
SC058	South Center	-	-	56-58	-	189
SC066	South Center	-	-	64-66	-	860
SC070	South Center	-	-	68-70	-	683
SC078	South Center	-	-	76-78	-	274
SC086	South Center	-	-	84-86	-	318
SC090	South Center	-	-	88-90	-	324
SC098	South Center	-	-	96-98	-	207
SC106	South Center	-	-	104-106	-	141
SC122	South Center	-	-	120-122	-	68
SH002	Shaokatan	44.41	-96.36	0-2	65	4566
SJ002	Saint James	43.98	-94.65	0-2	58	3320
SO002	St. Olaf	43.90	-93.42	0-2	55	590
ST002	South Twin	47.24	-95.65	0-2	43	4265
TR002	Trout	47.87	-90.17	0-2	31	3542
WI002	WhiteIron	47.89	-91.77	0-2	46	4293

Table 5.2. Inference using AlgaeBase to harmonize higher level taxonomy between the Diat.barcode v7 and Silva v138 genomic databases, which were used as references for *rbc*L and 18S amplicon sequence variant identification.

Diat.barcode v7 (*rbcL*)	Silva v.138 (18S)	Algae Base (Guiry et al. 2014)
Coscinodiscophyceae	Coscinodiscophytina_or	Coscinodiscophyceae
Mediophyceae	Mediophyceae_or	Mediophyceae
Fragilariophyceaea	Fragilariales	Fragilariales/Tabellariales
Bacillariophyceae	Bacillariophyceae_or	Bacillariales

Table 5.3. The total number of *rbc*L and 18S amplicon sequence variants matched for class, genus, and species taxonomic ranks from lake sediment DNA extractions.

Diat.barcode v7	# *rbc*L ASVs	Silva v138	# 18S ASVs
Coscinodiscophyceae	53	Coscinodiscophytina_or	24
Aulacoseira ambigua	3	*Aulacoseira*	24
A. granulata	13		
A. subarctica	1		
A. valida	1		
Melosira varians	1		
Uroselenia eriensis	6		
Unclassified	28	Unclassified	0
Mediophyceae	62	Mediophyceae_or	54
Cyclostephanos invisitatus	1	*Cyclotella*	10
C. tholiformis	4	*Discostella*	1
Cyclotella atomus	2	*Stephanodiscus*	9
C. cryptica	1	*Thalassiosira*	2
Discostella stelligera	1		
Lindavia bodanica	1		
Pantocsekiella costei	1		
Stephanodiscus hantzschii	7		
S. minutulus	2		
S. suzukii	1		
S. yellowstonensis	11		
Unclassified	30	Unclassified	32
Fragilariophyceaea	156	Fragilariales	62
Asterionella formosa	3	*Fragilaria*	6
Fragilaria gracilis	1	*Staurosira*	49
F. nanoides	1		
F. radians	3		
Pseudostaurosira brevistriata	4		
P. elliptica	5		
Staurosira construens	1		
S. venter	10		
Staurosirella martyi	8		
Tabellaria flocculosa	2		
Unclassified	118	Unclassified	7
Bacillariophyceae	339	Bacillariophyceae_or	100

Supplemental Table 5.1. Sample code and the number of the raw *rbc*L input sequences, and sequences remaining after the filter, denoising, merge, and removal of chimeric reads in the Callahan et al. (2016) *DADA2* pipeline.

Sample	input	filtered	denoisedF	denoisedR	merged	non-chimeric
AR002	42082	40872	40700	40775	40158	31684
BE002	41916	40707	40236	40384	38424	30354
CA002	39615	38376	37657	37850	33778	20066
CR002	26059	25134	24881	24857	23973	18075
HI002	12580	12010	11748	11807	11165	9991
HI004	42364	41096	40954	40866	39816	34659
HI006	35710	34611	34338	34452	32923	26376
HI008	38373	37197	36937	36932	35270	27483
HI010	40961	39682	39423	39526	38185	31467
HI012	43330	41977	41724	41846	40896	35640
HI014	41189	39926	39833	39692	39120	36282
HI016	20801	19537	19448	19440	19151	17813
HI018	41354	40121	40052	40077	39858	37598
HI022	16319	15621	15572	15574	15482	14765
HI024	33801	32863	32839	32820	32628	31000
HI030	33252	32224	32141	32152	31959	29284
HI032	28831	27888	27790	27826	27672	25338
HI038	23387	22434	22416	22394	22311	19816
MA002	35017	26507	25935	25796	21936	17252
PE002	35915	34702	34308	34327	33520	27221
PO002	31548	30405	30118	30125	29158	23425
PR002	32343	31237	30927	30971	29821	23007
RS002	32649	31504	31222	31183	30130	24625
SC002	34763	33586	32880	32975	30577	24897
SC006	30420	29503	29033	29044	27534	22430
SC010	32874	31776	31389	31305	29614	22607
SC018	33220	32097	31909	31811	31024	26290
SC038	39191	37821	37597	37692	36906	31072
SC046	4445	4253	4224	4224	4109	3805
SC058	2588	2500	2435	2409	2320	2121
SC066	14046	13181	13125	13159	13066	10810
SC070	26734	25850	25837	25731	25564	20751
SC078	34303	33200	33094	33135	32887	26996
SC086	8589	6751	6703	6694	6559	5657
SC090	24058	22973	22920	22900	22698	17725
SC106	18199	15446	15402	15374	15297	13045

SH002	35607	34585	34305	34332	33620	27642
SJ002	37448	36074	35573	35610	33698	26809
SO002	27842	26764	26360	26420	25182	20000
ST002	25364	24549	24061	24247	22956	18093
TR002	31854	30715	30222	30300	28131	19637
WI002	43737	42301	41758	41744	38780	29929

Supplemental Table 5.2. Sample code and the number of raw 18S input sequences, and sequences remaining after the filter, denoising, merge, and removal of chimeric reads in the Callahan et al. (2016) *DADA2* pipeline.

Sample	input	filtered	denoisedF	denoisedR	merged	non-chimeric
AR0	37769	34141	33578	33768	30933	27523
BE0	32420	29766	29153	29460	25145	20074
CA0	41736	37723	36764	37222	32033	28229
CR0	29626	26498	25934	26222	22319	20863
HI0	36444	32516	31838	32059	28122	25065
HI11	30908	28028	27792	27834	26485	25959
HI13	46449	40858	40664	40601	38814	38669
HI15	29442	26356	26161	26174	25215	25082
HI17	26788	23787	23671	23734	23252	23250
HI21	3945	3508	3424	3444	3224	3222
HI23	35761	32700	32612	32561	32043	31974
HI29	29824	26819	26714	26653	25999	25852
HI3	37783	33853	33390	33581	31205	30448
HI31	41205	37054	36839	36913	36261	36243
HI37	31860	27860	27701	27735	27203	27200
HI5	28879	26513	26193	26288	24166	22994
HI7	37777	34383	33994	34028	31391	30084
HI9	27770	25413	24956	25012	21635	19814
MA0	36149	28582	27811	28187	22734	15960
PE0	41339	37280	36608	36915	34585	33575
PO0	42018	38227	37759	37960	35619	34122
PR0	42121	38100	37307	37601	33189	29925
RS0	41756	37835	37205	37398	32842	32040
SC0	34123	29197	28348	28655	24182	20585
SC104	45642	41107	40941	40928	40221	40172
SC16	36248	32707	31969	32318	27980	23999
SC24	36200	31923	31618	31778	30980	30889
SC36	42999	38464	37844	38004	34931	29641
SC4	37173	32291	31644	31818	28630	26180
SC44	61852	55944	55685	55770	54955	54398
SC56	42324	38503	38270	38325	37585	37560
SC64	40134	35866	35673	35675	34852	34471
SC68	6439	5813	5677	5671	5219	4795
SC76	31882	27527	27355	27403	26760	26315
SC8	38686	34736	33912	34311	30248	26818
SC84	31475	12779	12593	12183	11115	11092

SC88	35014	31433	31183	31340	30650	30571
SH0	37544	34149	33498	33771	30564	27343
SJ0	36874	33007	32324	32569	28075	23870
SO0	43464	39530	38795	39041	35024	25608
ST0	24144	21441	20986	21206	19079	18796
TR0	29032	26520	25942	26171	21983	19355
WI0	27609	25037	24342	24648	20945	19870

Supplemental Table 5.3. (Ch5.rbcLtaxonomy.csv) The amplicon sequence variant (ASV) identification number, and taxonomic assignment (Empire, Kingdom, Subkingdom, Phylum, Class, Order, Family, Genus, & Species) for 1,943 diatoms identified using *rbc*L ASVs from lake sediments.

Supplemental Table 5.4. (Ch5.18Staxonomy.csv) The amplicon sequence variant (ASV) identification number, and taxonomic assignment (Kingdom, Phylum, Class, Order, Family, & Genus) for 311 diatoms identified using 18S ASVs from lake sediments.

Figures

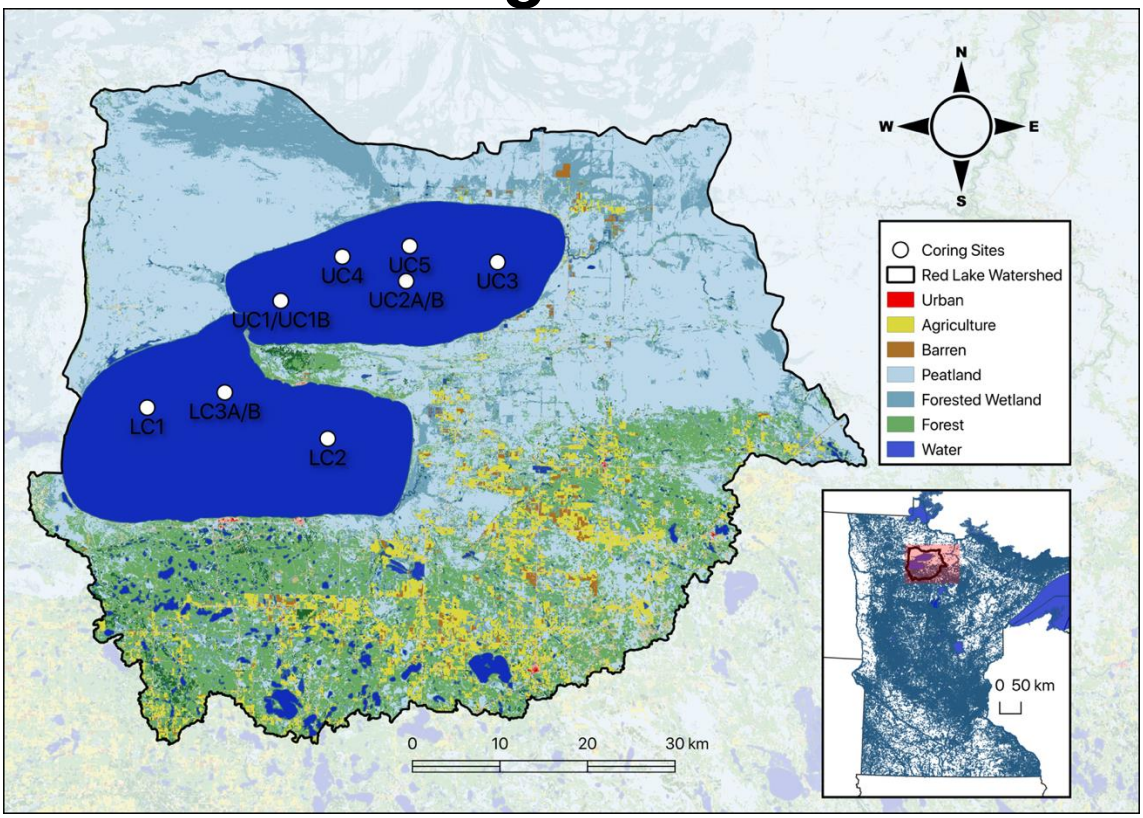


Figure 2.1. Site map showing the Upper and Lower Red Lakes coring sites, lakes, outflow, land-use, and location inset.

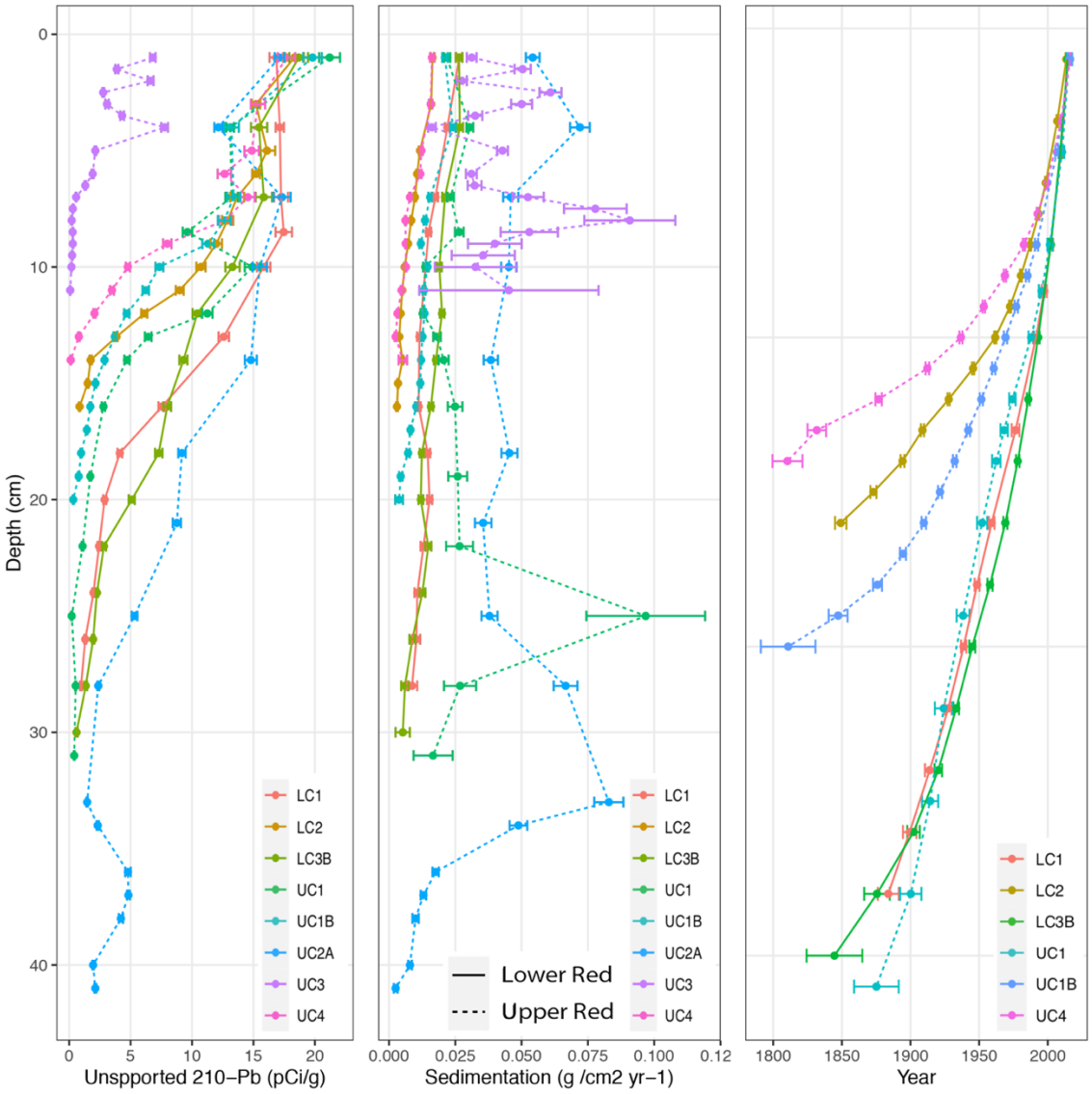


Figure 2.2. Unsupported ^{210}Pb, sedimentation rate, and ^{210}Pb year estimate plotted by sediment core depth on the y-axis for sediment cores recovered from Upper Red Lake (dashed line) and Lower Red Lake (solid line). Error bars based on propagation of counting uncertainty.

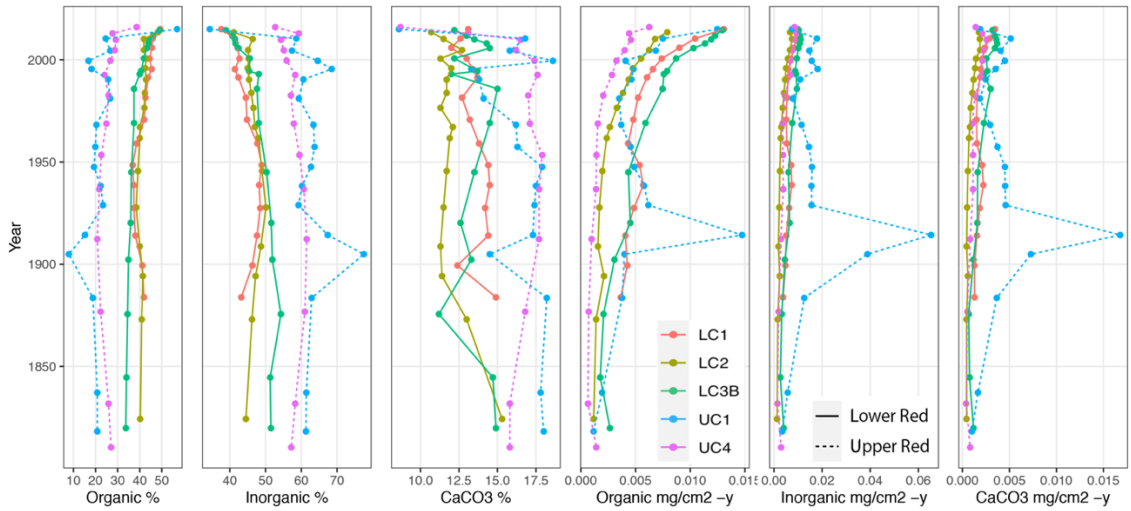


Figure 2.3. The percentage and flux of organic, inorganic, and calcium carbonate for Upper Red Lake (dashed line) and Lower Red Lake (solid line) sediment cores plotted by year on the y-axis.

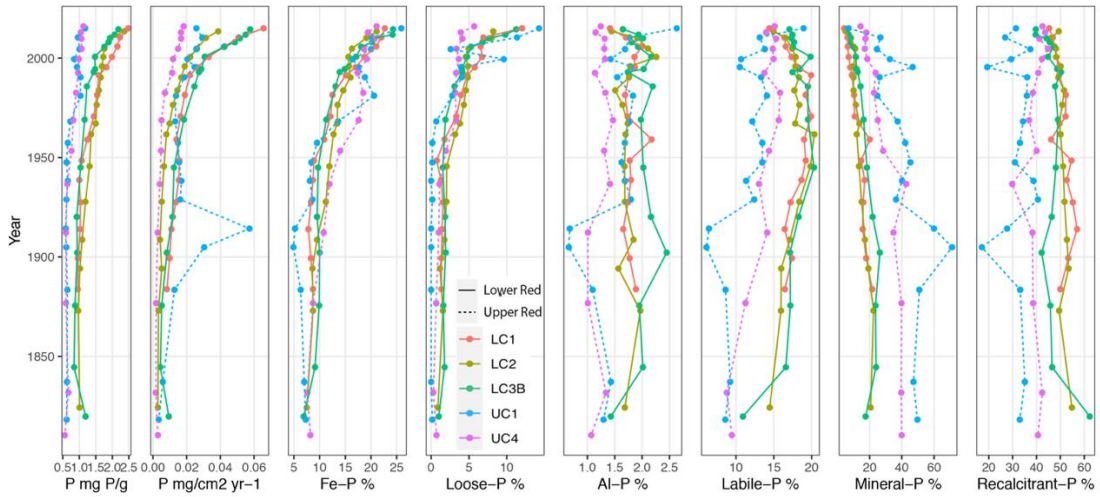


Figure 2.4. Sediment phosphorus concentration and accumulation rate, and the percent Fe-P, loose-P, Al-P, labile-P, mineral-P, and recalcitrant-P, plotted by estimated sediment year on the y-axis for Upper Red Lake (dashed line) and Lower Red Lake (solid line).

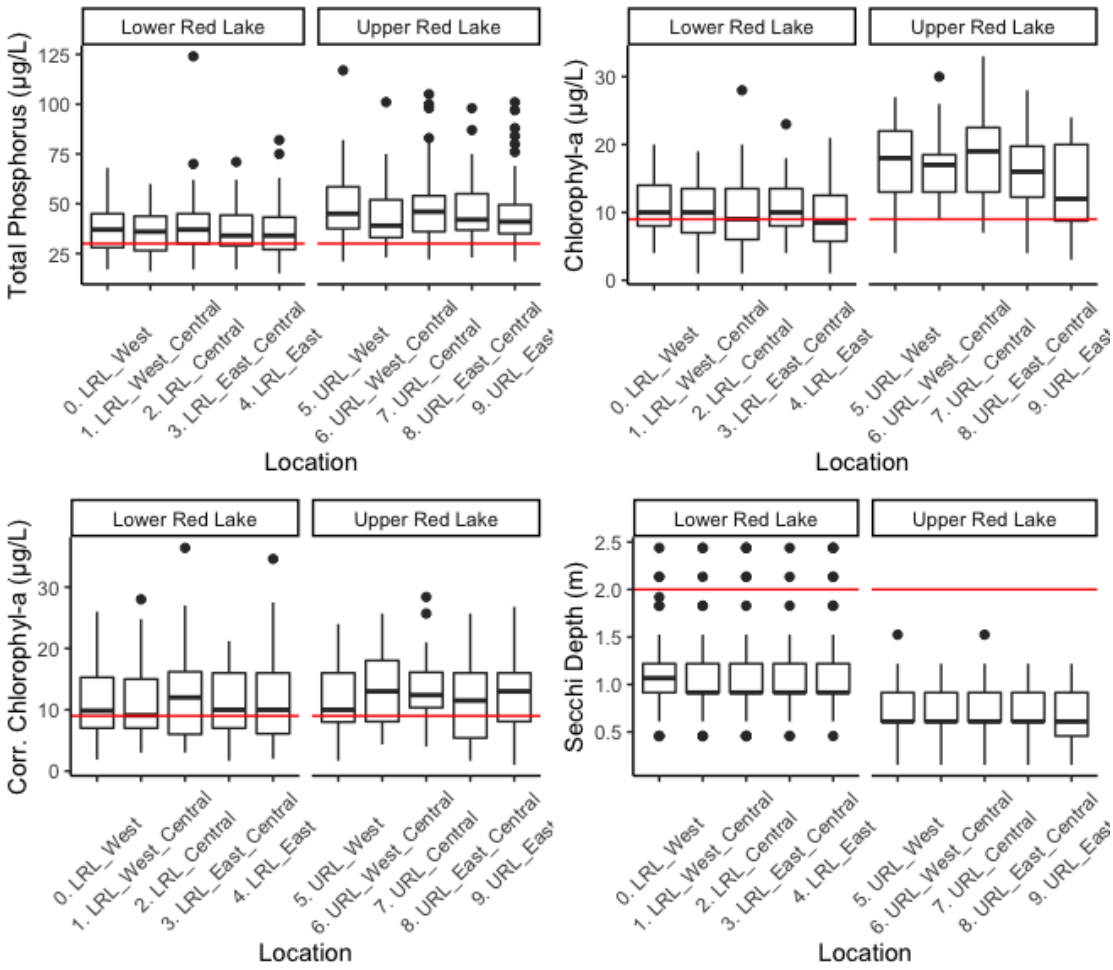


Figure 2.5. Box plots of monitored total phosphorus (μg L^{-1}), chlorophyll-a (μg L^{-1}), phaeophytin corrected chlorophyll-a (μg L^{-1}), and Secchi depth (m) for five sampling stations in Lower Red Lake (left) and Upper Red Lake (right). The solid red lines on each plot represent the Minnesota Pollution Control Agency Northern Lakes and Forests Ecoregion nutrient criteria for total phosphorus, chlorophyll-a, and Secchi depth.

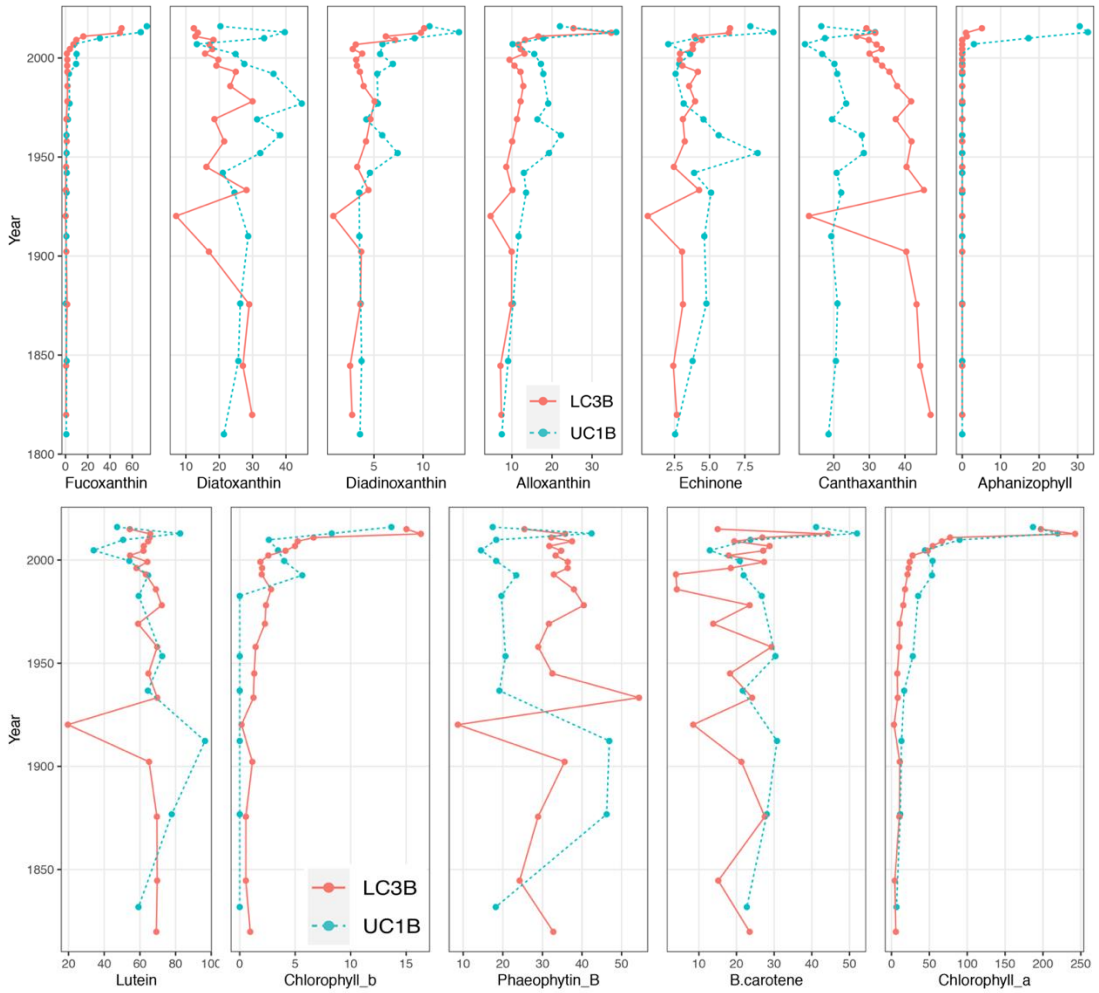


Figure 2.6. Algal pigment concentrations (nmol/g C) plotted by year from Upper Red Lake sediment core 1B (solid line) and Lower Red Lake sediment core 3B (dashed line).

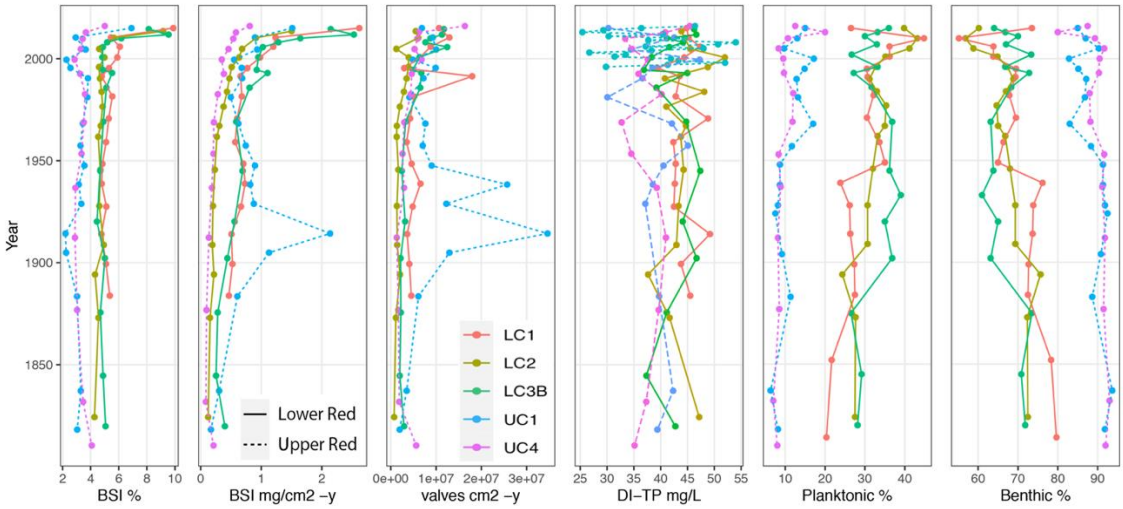


Figure 2.7. The percent and flux of biogenic Si, diatom valve density, diatom-inferred total phosphorus, and the percentage of planktonic and benthic diatom taxa from the Upper Red Lake sediment cores UC1 and UC4 (dashed lines) and Lower Red Lake sediment cores LC1, LC2, and LC3B (solid lines).

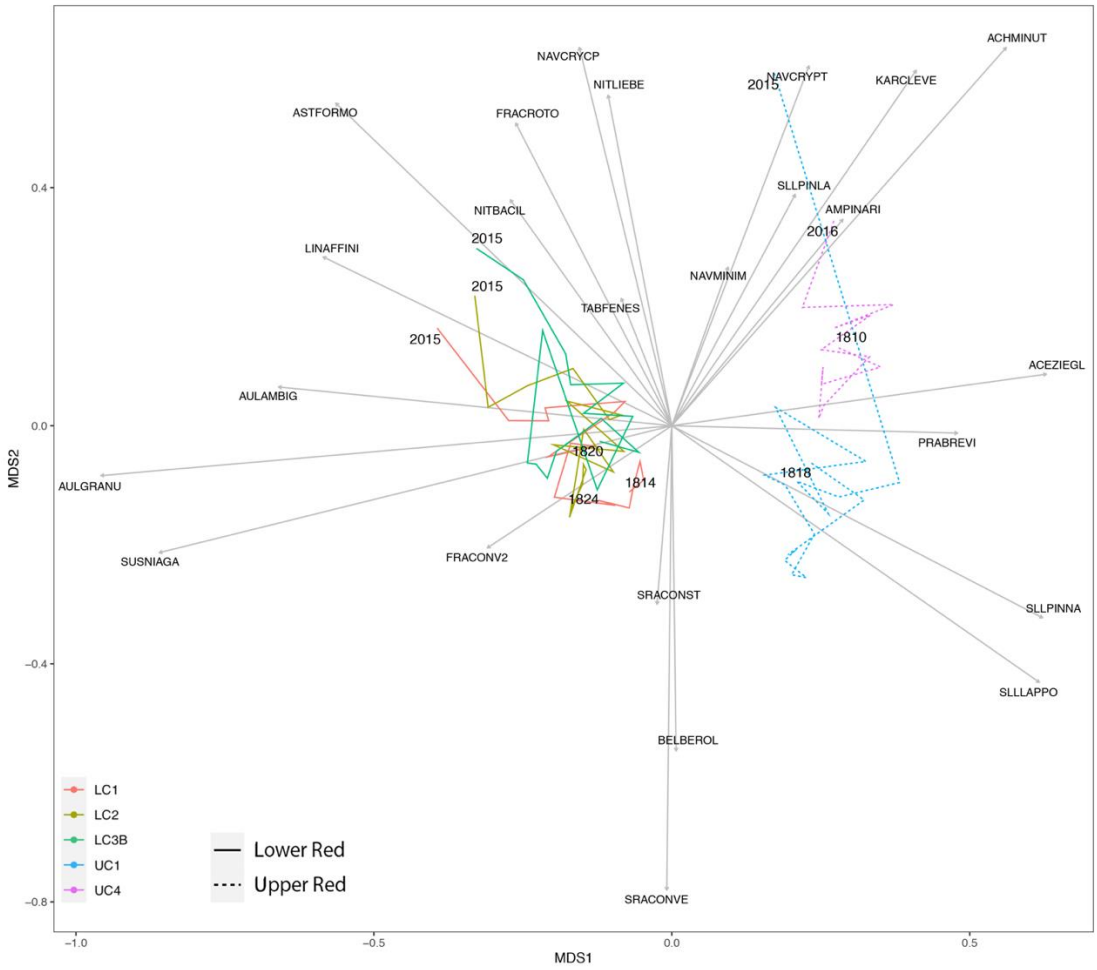


Figure 2.8. Two-dimensional solution for a nonmetric multi-dimensional scaling ordination of common diatoms observed in the Upper Red Lake (dashed lines) and Lower Red Lake (solid lines) sediment cores (stress = 0.14, non-metric stress r^2 = 0.98).

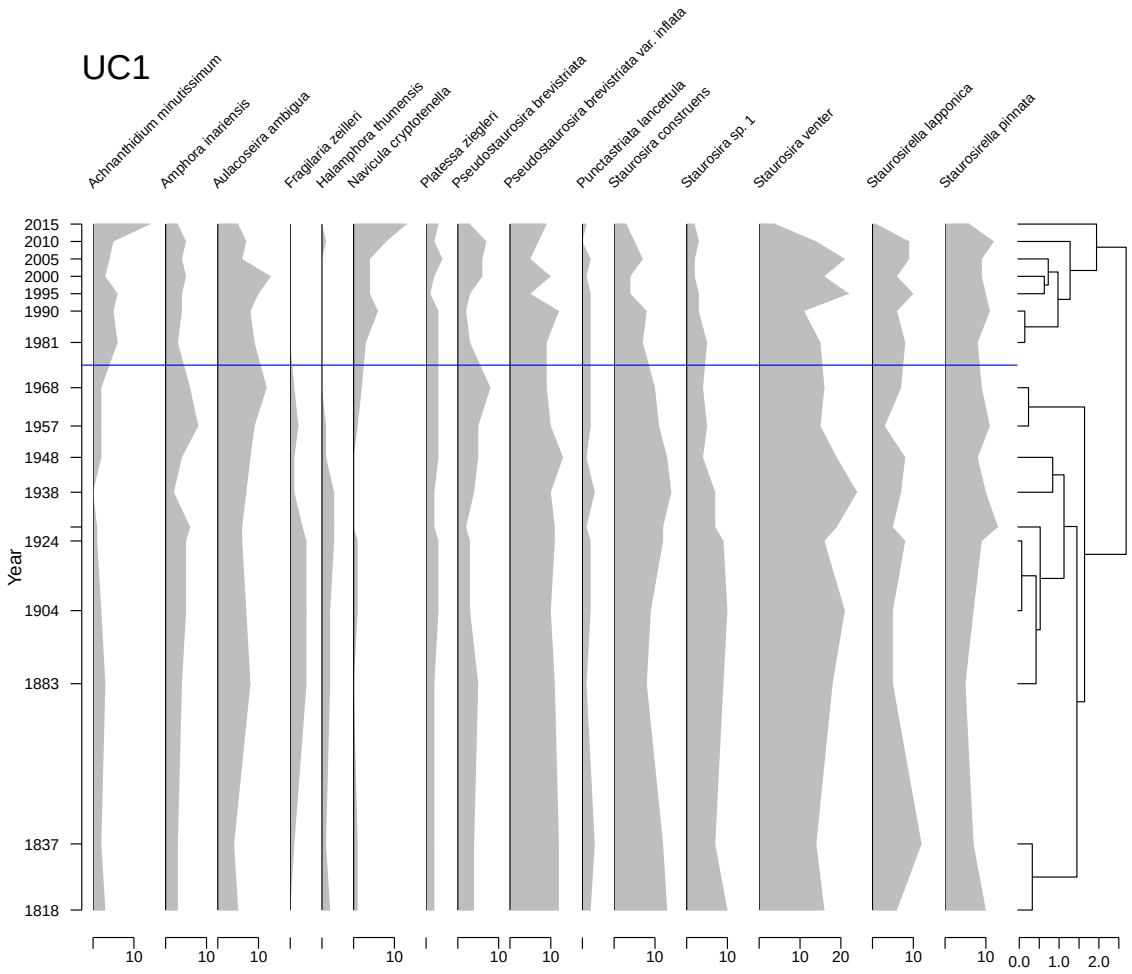


Figure 2.9. Stratigraphic plot of the dominant diatoms, as percent relative abundance, from the Upper Red Lake sediment core #1 plotted by ^{210}Pb estimated year on the y-axis. The solid blue line represents a significant break in the constrained cluster analysis of diatom assemblages shown on the right.

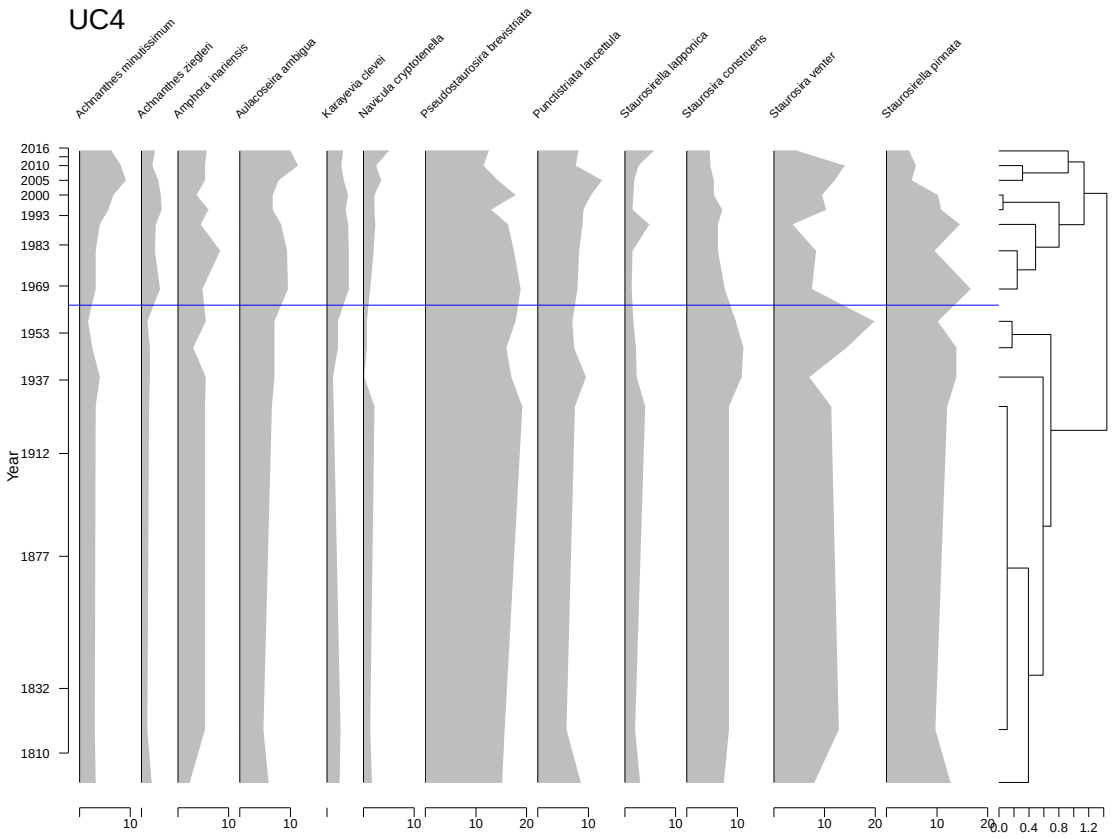


Figure 2.10. Stratigraphic plot of the dominant diatoms, as percent relative abundance, from the Upper Red Lake sediment core #4 plotted by ^{210}Pb estimated year on the y-axis. The solid blue line represents a significant break in the constrained cluster analysis of diatom assemblages shown on the right.

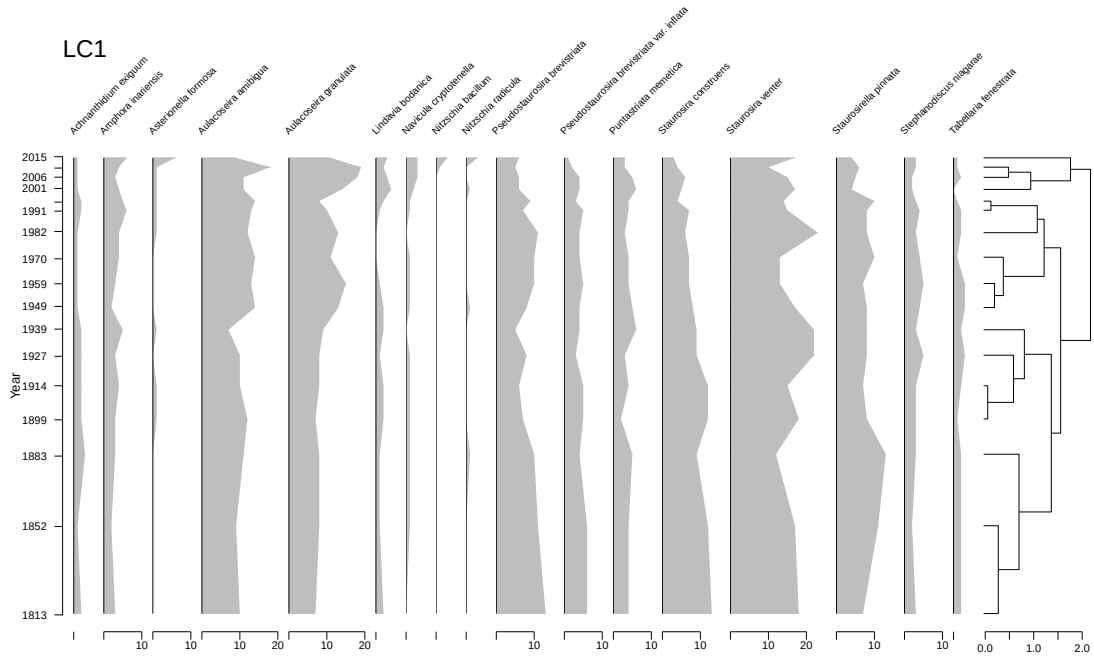


Figure 2.11. Stratigraphic plot of the dominant diatoms, as percent relative abundance, from the Lower Red Lake sediment core #1 plotted by ^{210}Pb estimated year on the y-axis. A constrained cluster analysis of diatom assemblages shown on the right.

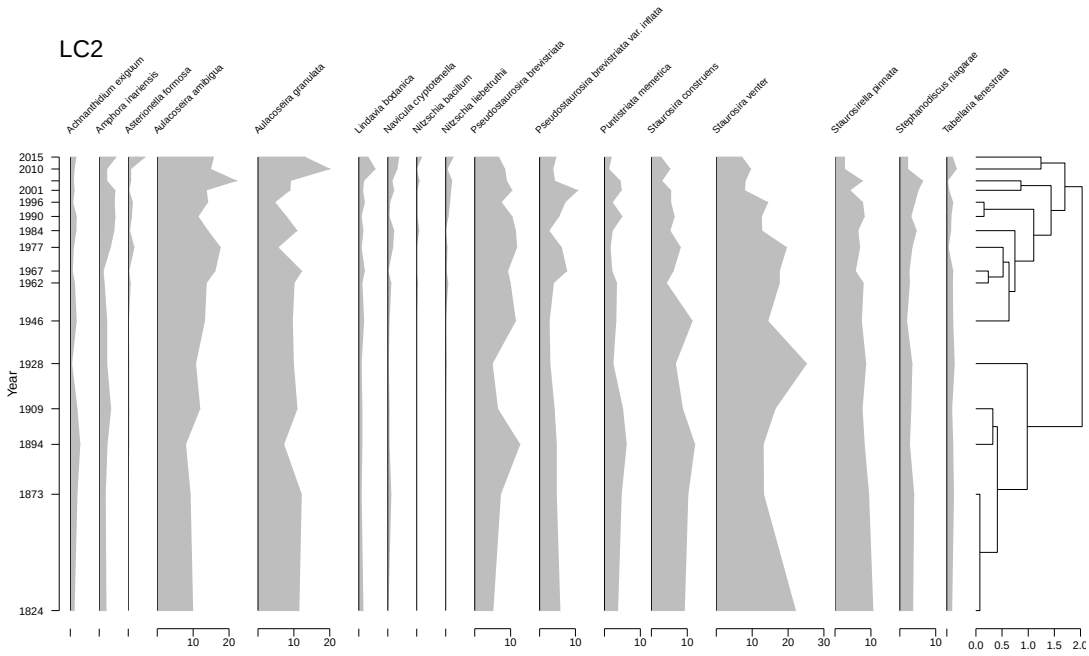


Figure 2.12. Stratigraphic plot of the dominant diatoms, as percent relative abundance, from the Lower Red Lake sediment core #2 plotted by ^{210}Pb estimated year on the y-axis. A constrained cluster analysis of diatom assemblages shown on the right.

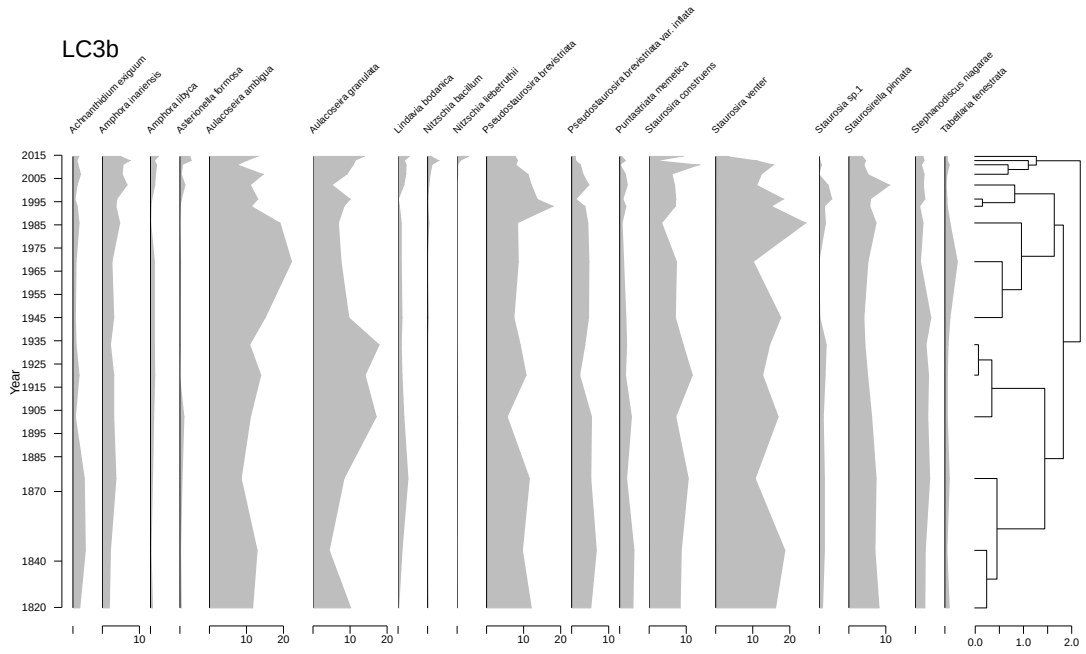


Figure 2.13. Stratigraphic plot of the dominant diatoms, as percent relative abundance, from the Lower Red Lake sediment core #3B plotted by ^{210}Pb estimated year on the y-axis. A constrained cluster analysis of diatom assemblages shown on the right.

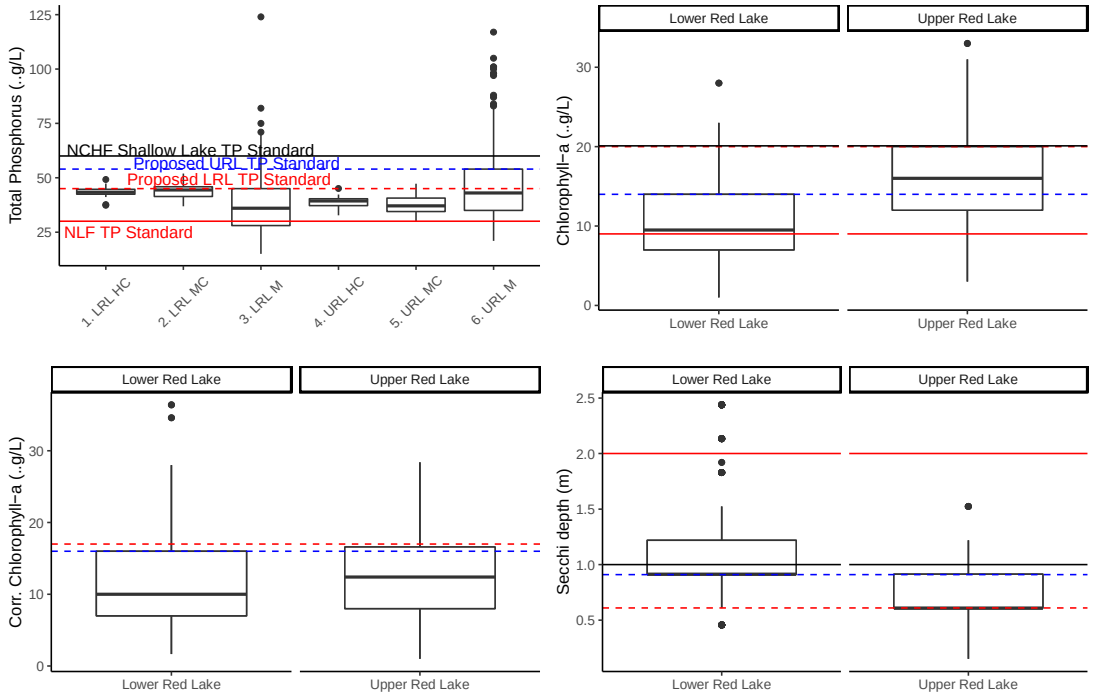
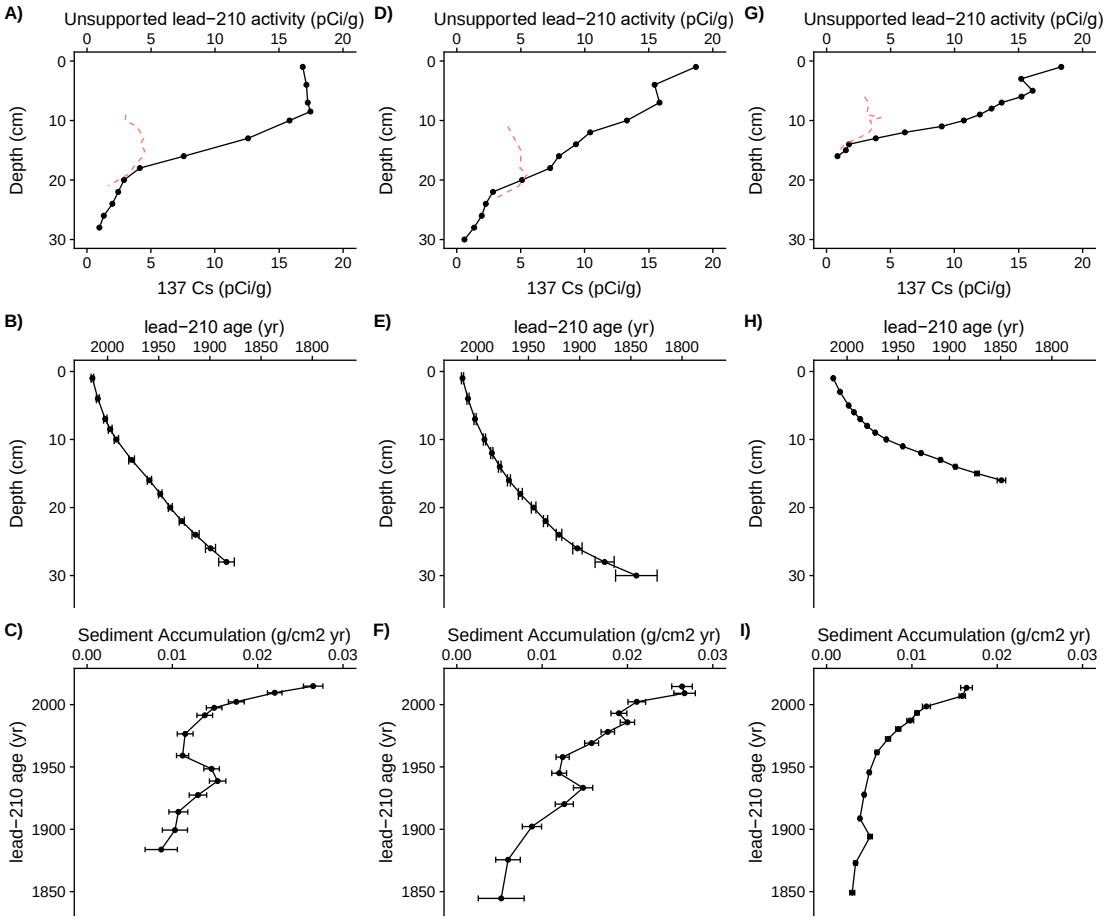
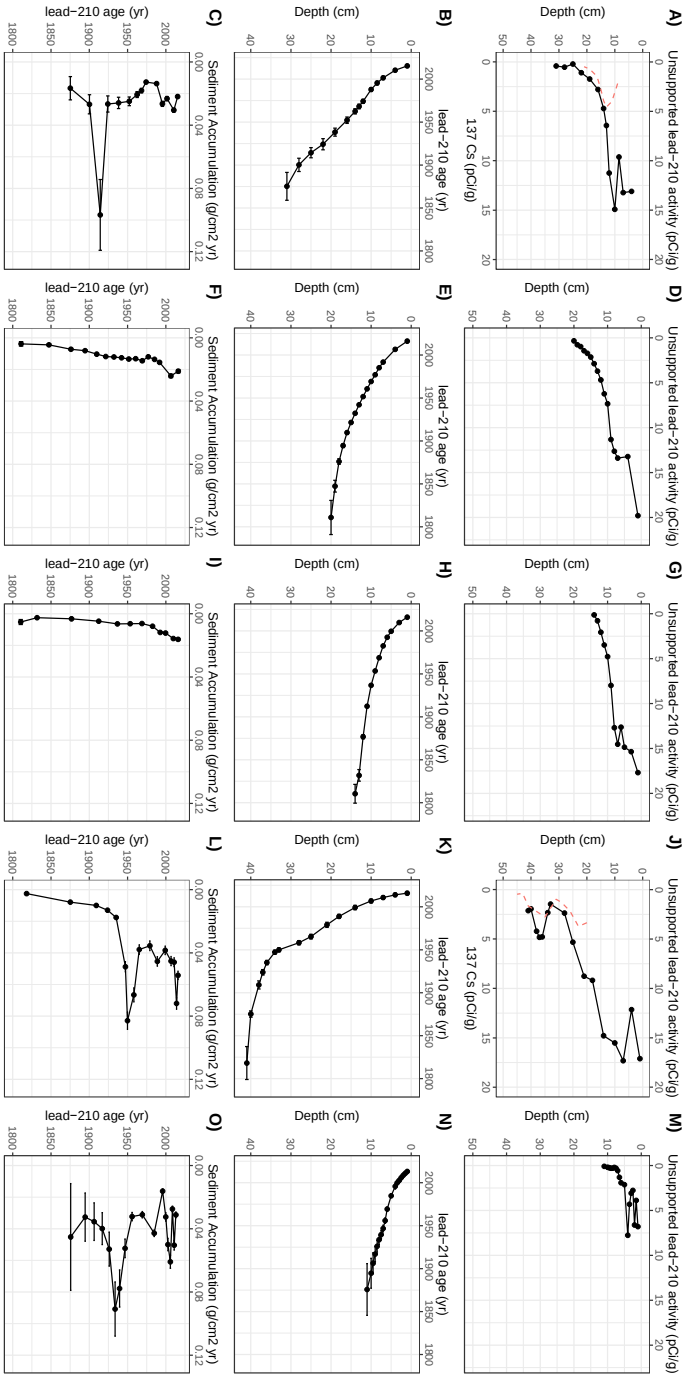


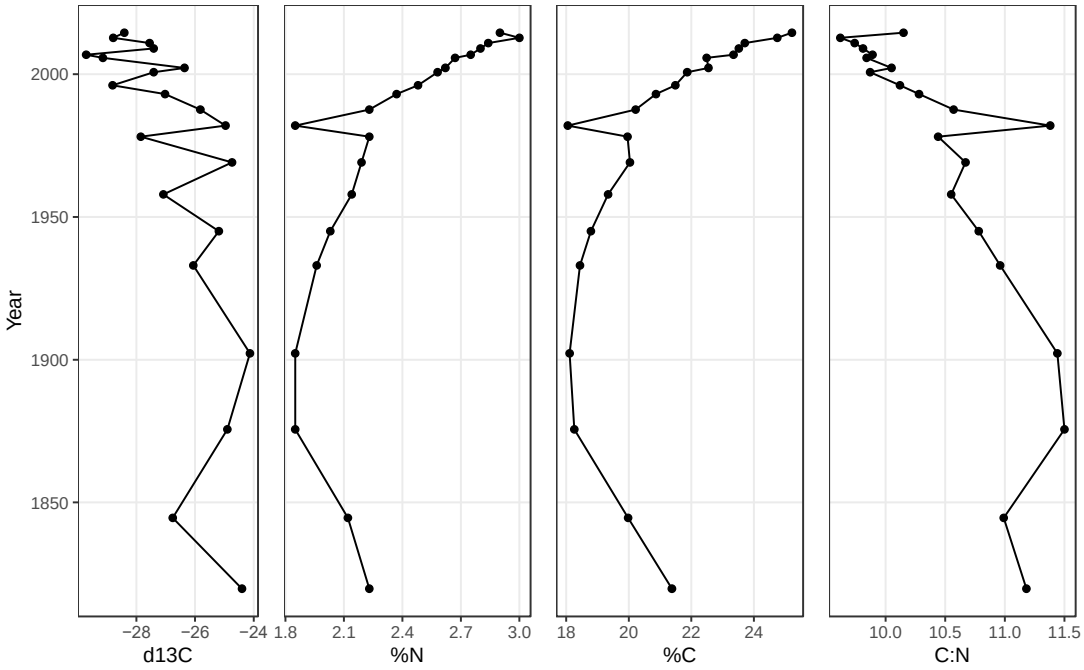
Figure 2.14. Box plots of monitored total phosphorus (μg L^{-1}), modern diatom-inferred total phosphorus (μg L^{-1}), historical diatom-inferred total phosphorus (μg L^{-1}), chlorophyll-a (μg L^{-1}), phaeophytin corrected chlorophyll-a (μg L^{-1}), and Secchi depth (m) averaged for Lower Red Lake (left) and Upper Red Lake (right). The solid red lines on each plot represent the Minnesota Pollution Control Agency Northern Lakes and Forests Ecoregion nutrient criteria for total phosphorus, chlorophyll-a, and Secchi depth and the solid black lines represent the Central Hardwood Forests ecoregion standards. The dashed blue line represents the proposed standards for Lower Red Lake and the dashed red line represents the proposed standards for Upper Red Lake.



Supplemental figure 2.1. Unsupported ^{210}Pb, ^{137}Cs (red dashed line), and ^{210}Pb year estimate plotted by depth, and the sediment accumulation rate plotted by year for Lower Red Lake sediment cores LC1 (A-C), LC2 (D-F), and LC3B (G-I).



Supplemental figure 2.2 Unsupported ^{210}Pb, ^{137}Cs (red dashed line), and ^{210}Pb year estimate plotted by depth, and the sediment accumulation rate plotted by year for Upper Red Lake sediment cores UC1 (A-C), UC4 (D-F), UC5 (G-I), UC2A (J-L), and UC3 (M-O).



Supplemental figure 2.3 Plotted by ^{210}Pb age on the y-axis are the values for $\delta^{13}C$, Nitrogen (%), Carbon (%), and Carbon: Nitrogen for the LC3B sediment core.

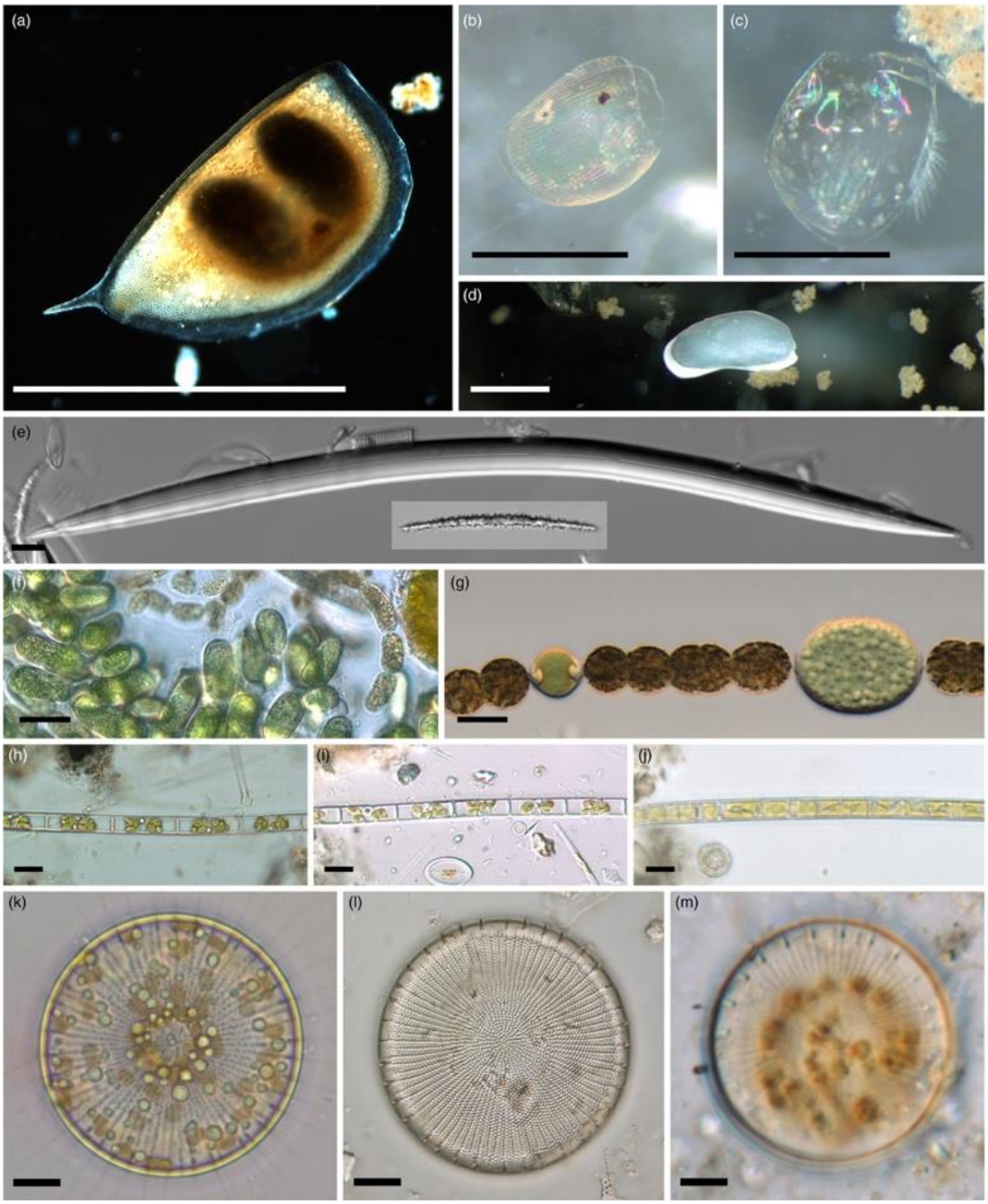


Figure 3.1 Light micrographs of dormant propagules and sediment microfossils. (a) *Daphnia pulicaria* ephippium with two dormant eggs (b, c) cladoceran exoskeletons (d) ostracod shell (e) freshwater sponge megasclere with smaller gemmosclere spicule (inset) (f) the cyanobacterium *Dolichospermum* sp. with mass production of akinetes (g) short filament of *Dolichospermum* sp. showing translucent heterocyte and larger akinete (h, i) filaments with dormant cells of the diatom *Aulacoseira ambigua* (j) rejuvenated cell of *A. ambigua* (k) live vegetative cell of the diatom *Stephanodiscus niagarae* (l) single valve of *S. niagarae* preserved in sediment (m) dormant resting cell of *S*. *niagarae.* Scale bars = 10 µm (e-m); 1 mm (a, d); 0.5 mm (b, c).

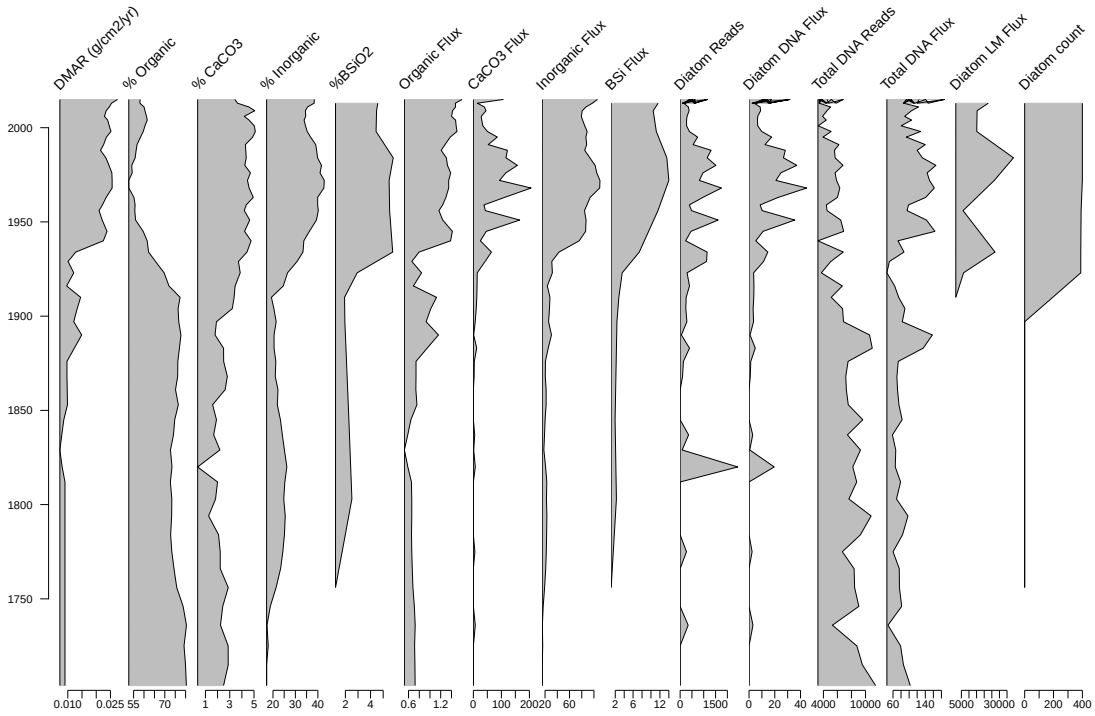


Figure 4.1. Biogeochemical parameters dry mass accumulation rate (DMAR, g cm^{-2} yr^{-1}), the percentage (%), and flux (g cm^{-2} yr^{-1}) for organic, $CaCO_3$, inorganic, and BSi sediment components, the count and flux of diatom frustules, and the reads and relative fluxes for the total and diatom 23S DNA) plotted by ^{210}Pb year (C.E.) from Cedar Bog Lake sediments.

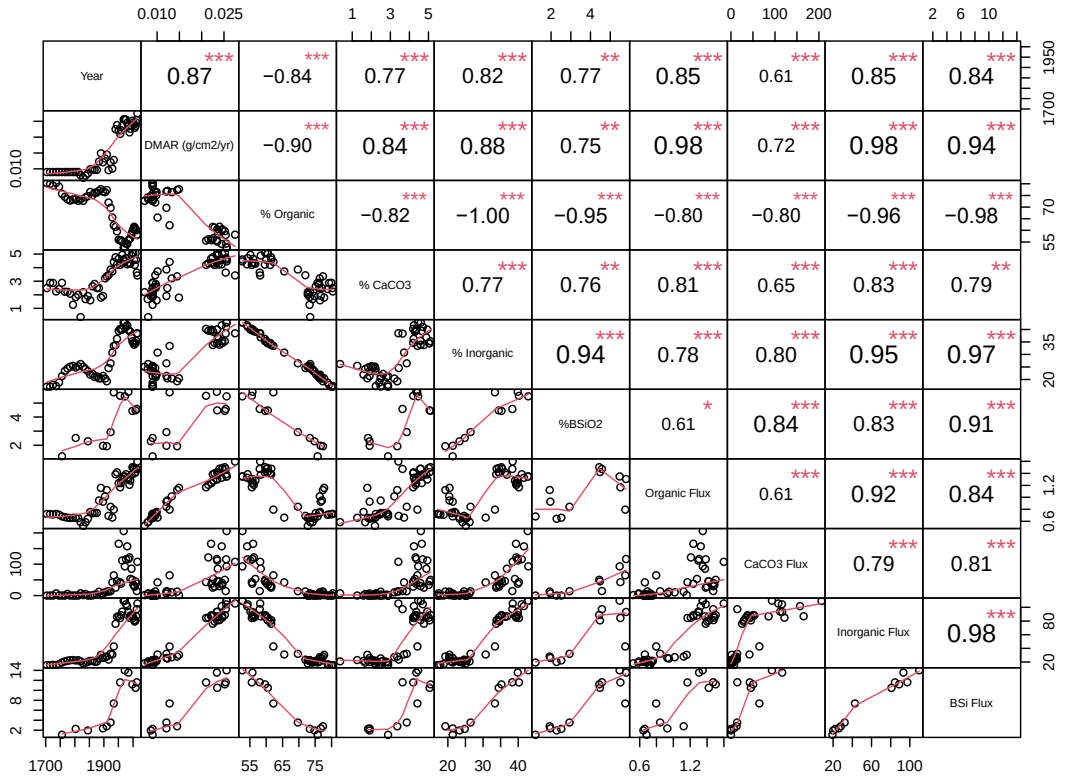


Figure 4.2. A Pearson's correlation matrix of Cedar Bog Lake geochemistry data descending diagonally to the right on the graph with labeled parameters represented by histograms for the following parameters: year, dry mass accumulation rate (DMAR), percent organic, $CaCO_3$, inorganic, and biogenic Si (BSi) by weight, and organic, $CaCO_3$, inorganic, and BSi flux (g cm^{-2} yr^{-1}). Linear trends and Pearson's correlation values are plotted at the conjuncture of values on the bottom left and top right respectively. The p-values 0.001, 0.01, and 0.05 are represented by symbols "***", "**", and "*" respectively.

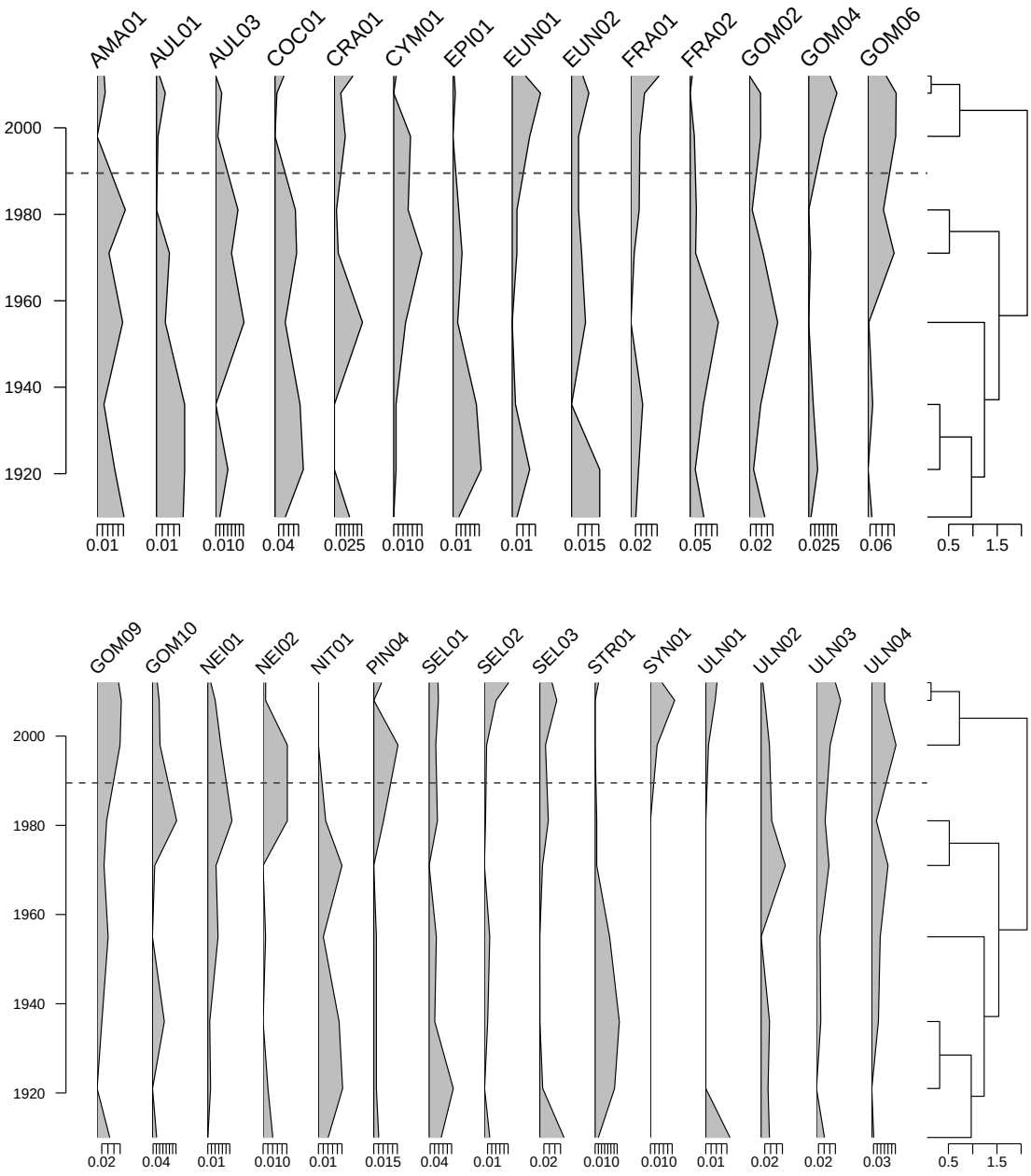


Figure 4.3. The relative abundance of diatoms detected using light microscopy plotted by ^{210}Pb year (C.E.). Diatoms identified by their voucher flora code (see Table 3); taxa present at ≥ 3% abundance in ≥ 3 samples in the Cedar Bog Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.

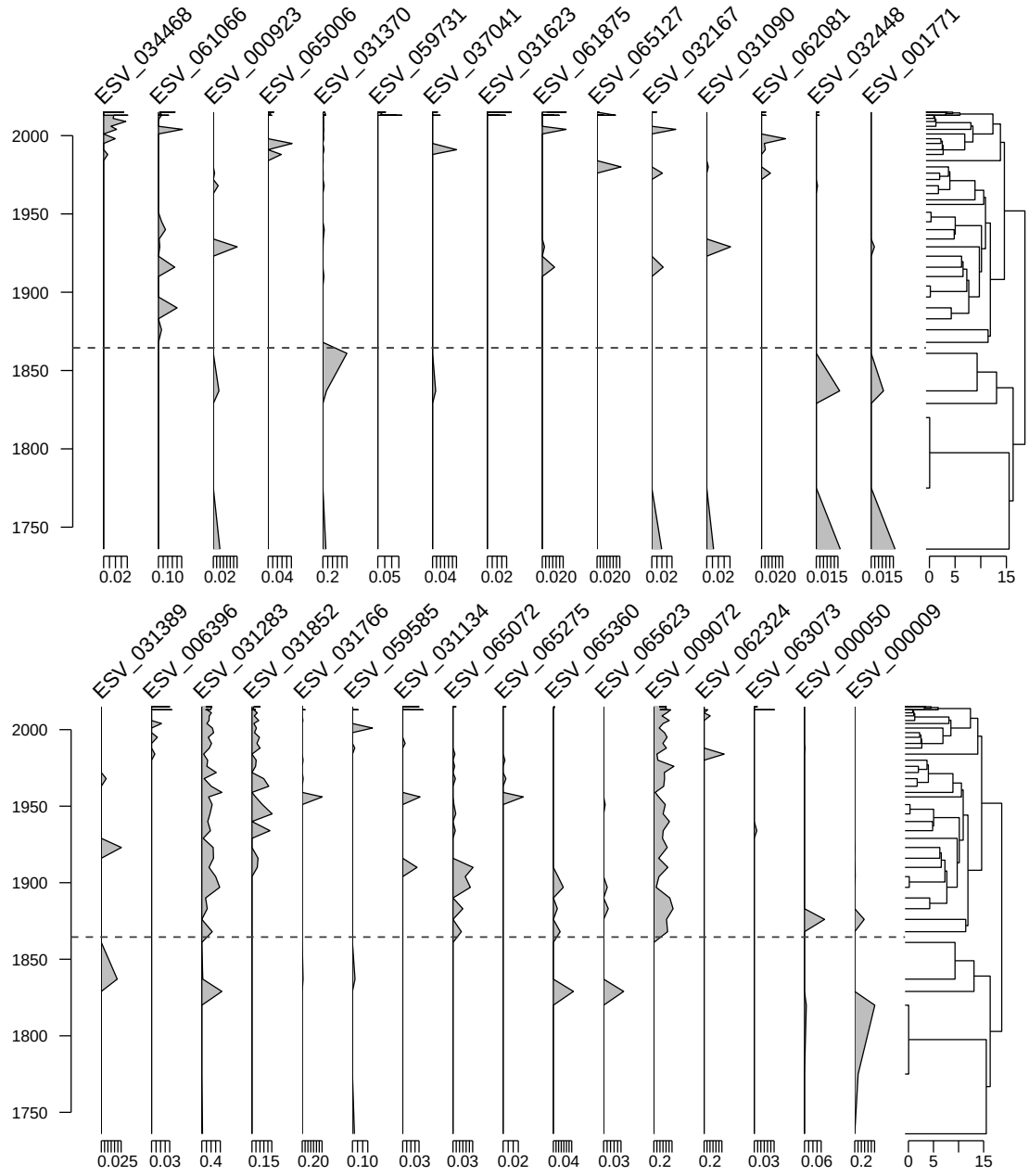


Figure 4.4. The relative abundance of diatoms detected by 23S amplicon sequencing plotted by ^{210}Pb year (C.E.). Diatoms identified by their exact sequence variant identification code (see Table 2); taxa present at ≥ 3% abundance in ≥ 3 samples in the Cedar Bog Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.

Supplemental Figure 4.1. Cedar Bog Lake voucher flora used for identification consistency. Taxa are identified by a 3-letter genus code followed by a two-digit number for each newly encountered species within that genus. Generic codes are defined as AMA = *Amphora*, AUL = *Aulacoseira*, CMB = *Cymbopleura*, COC = *Cocconeis*, CRA = *Craticula*, ENC = *Encyonema*, EPI = *Epithemia*, EUN = *Eunotia*, FRA= *Fragilaria/Fragilariforma*, GOM = *Gomphonema*, MER = *Meridion*, NEI = *Neidium*, NIT = *Nitzschia*, PIN = *Pinnularia*, REX = *Rexlowea*, SEL = *Sellaphora*, STA = *Stauroneis*, and ULN = *Ulnaria*. Scale bars are represented by solid black squares representing 10 *μ*m. (Cedar Bog Lake Fora.jpg).

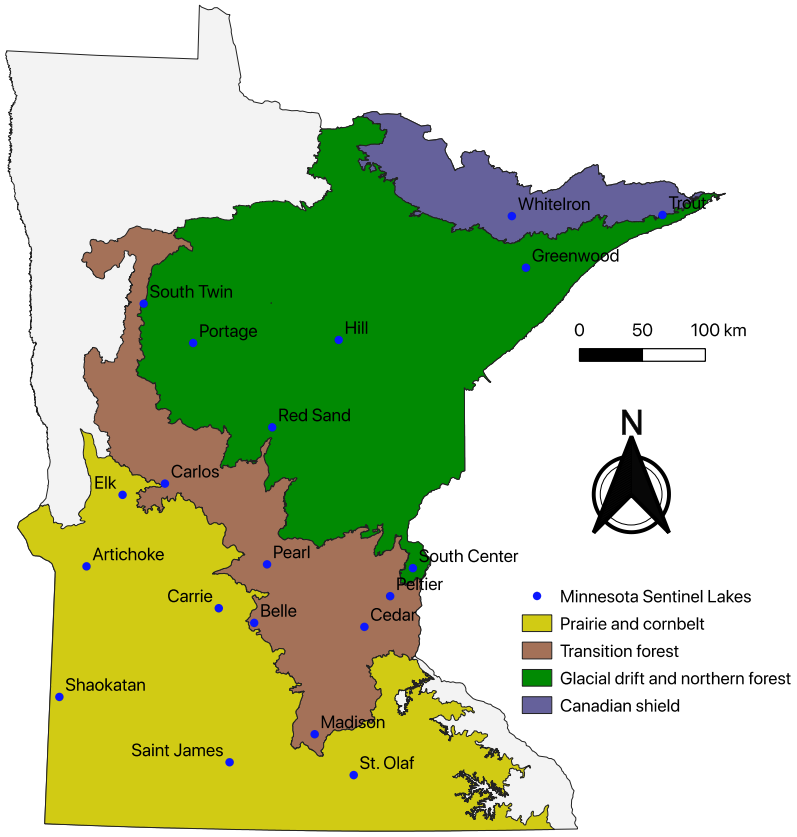


Figure 5.1. The distribution of Minnesota Department of Natural Resources Sentinel Lakes and ecoregions, where sediments were examined for bulk DNA characterization of diatom assemblages.

Figure 5.2. Results for 50 samples and UMNGC quality control blanks for A) the amount of DNA extracted (ng), B) raw sequence read count from Illumina sequencing, C) filtered non-chimeric sequence read count, and D) number of amplicon sequence variants. Circles represent sediment core samples, triangles represent quality control blanks, and squares represent surface sediment samples. Amplicons are color coded, blue for *rbc*L, and red for 18S.

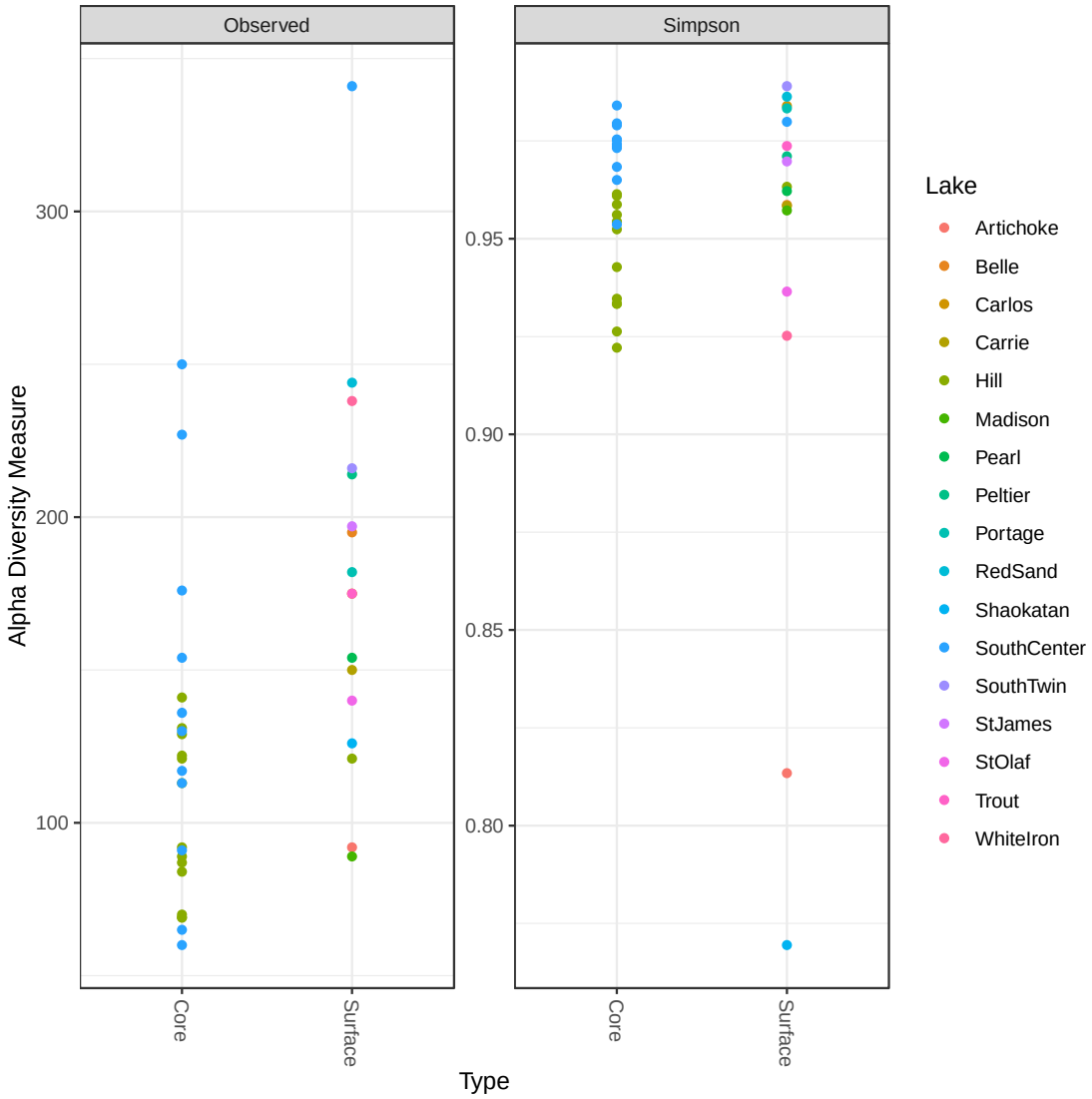


Figure 5.3. The alpha (Observed) and Simpson's diversity diatom amplicon sequence variants detected using *rbc*L for Hill and South Center Lake sediment cores and surface sediment samples from other Minnesota lakes.

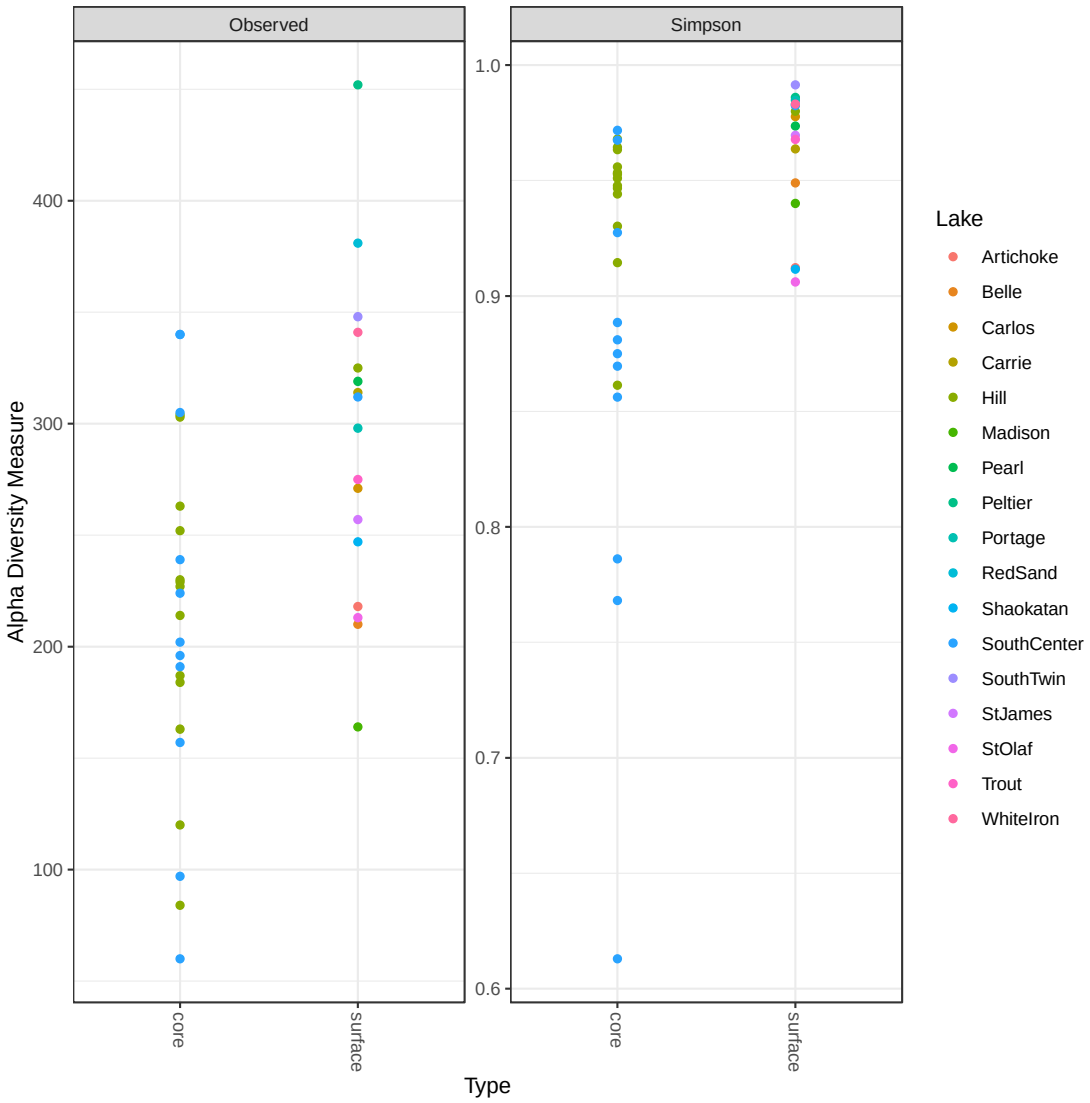


Figure 5.4. The alpha (Observed) and Simpson's diversity diatom amplicon sequence variants detected using 18S for Hill and South Center Lake sediment cores and surface sediment samples from other Minnesota lakes.

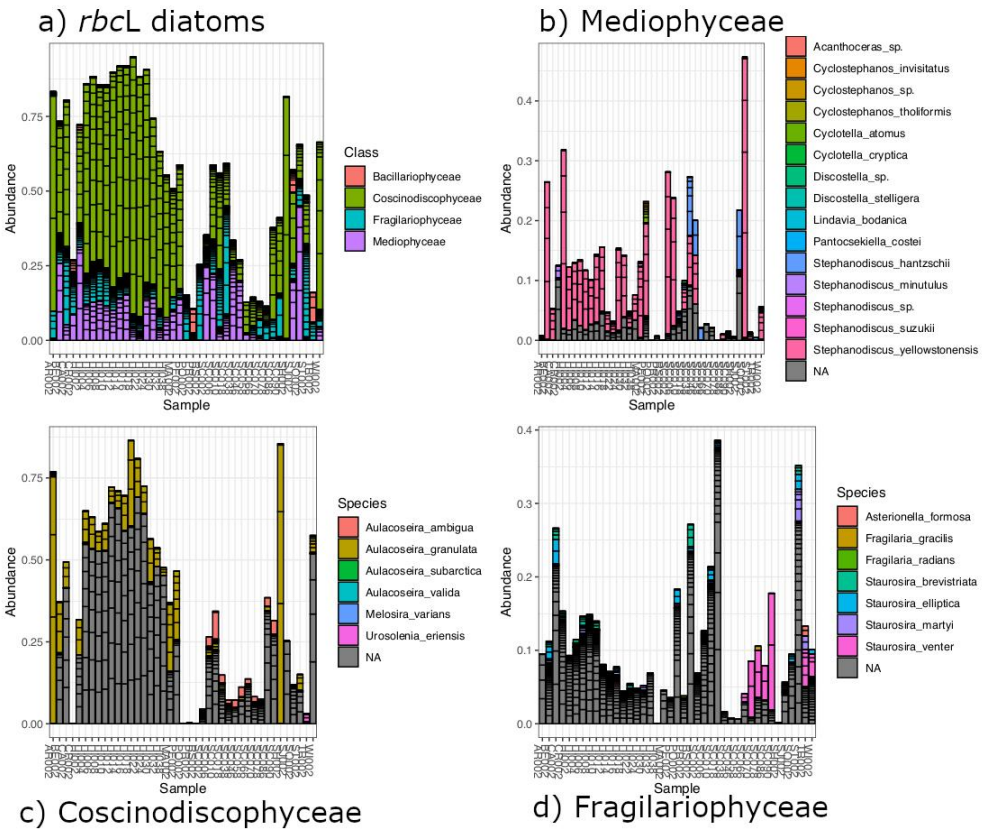


Figure 5.5. Sample relative read abundance for diatom *rbc*L amplicon sequence variants by a) taxonomic class, and within b) Mediophyceae, c) Coscinodiscophyceae, and d) Fragilariophyceae according to Diat.barcode v7.

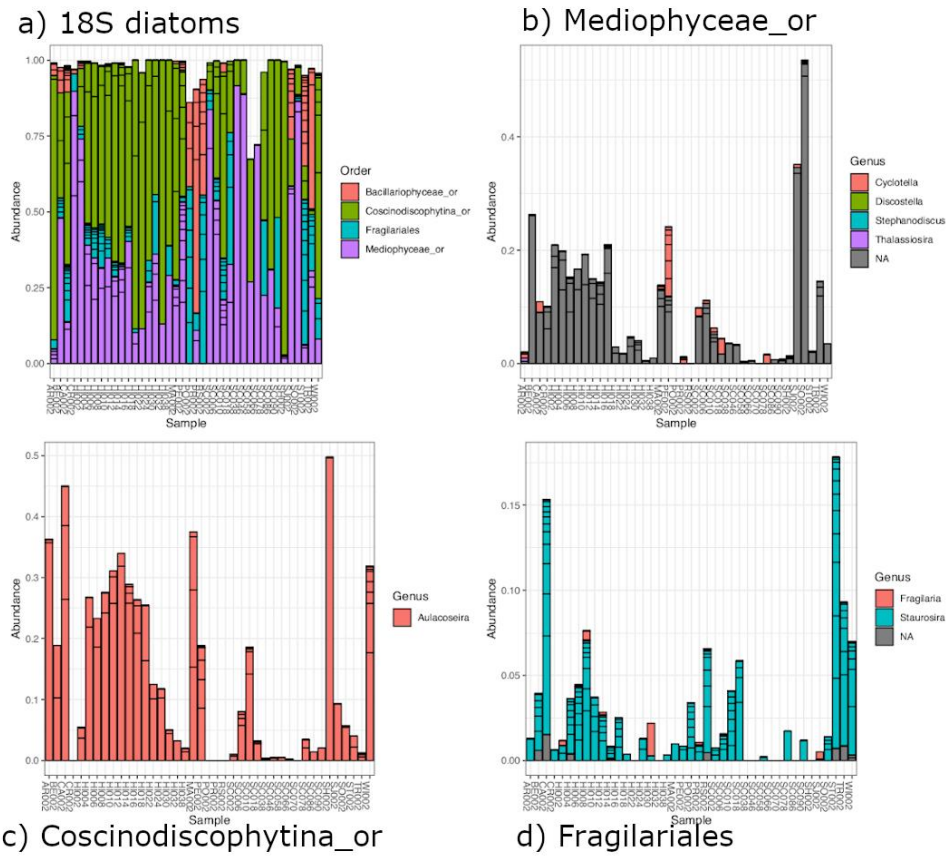


Figure 5.6. Sample relative read abundance for diatom 18S amplicon sequence variants by a) taxonomic class, and within b) “Mediophyceae_or”, c) “Coscinodiscophytina_or”, and d) “Fragilariales” according to Silva v138.

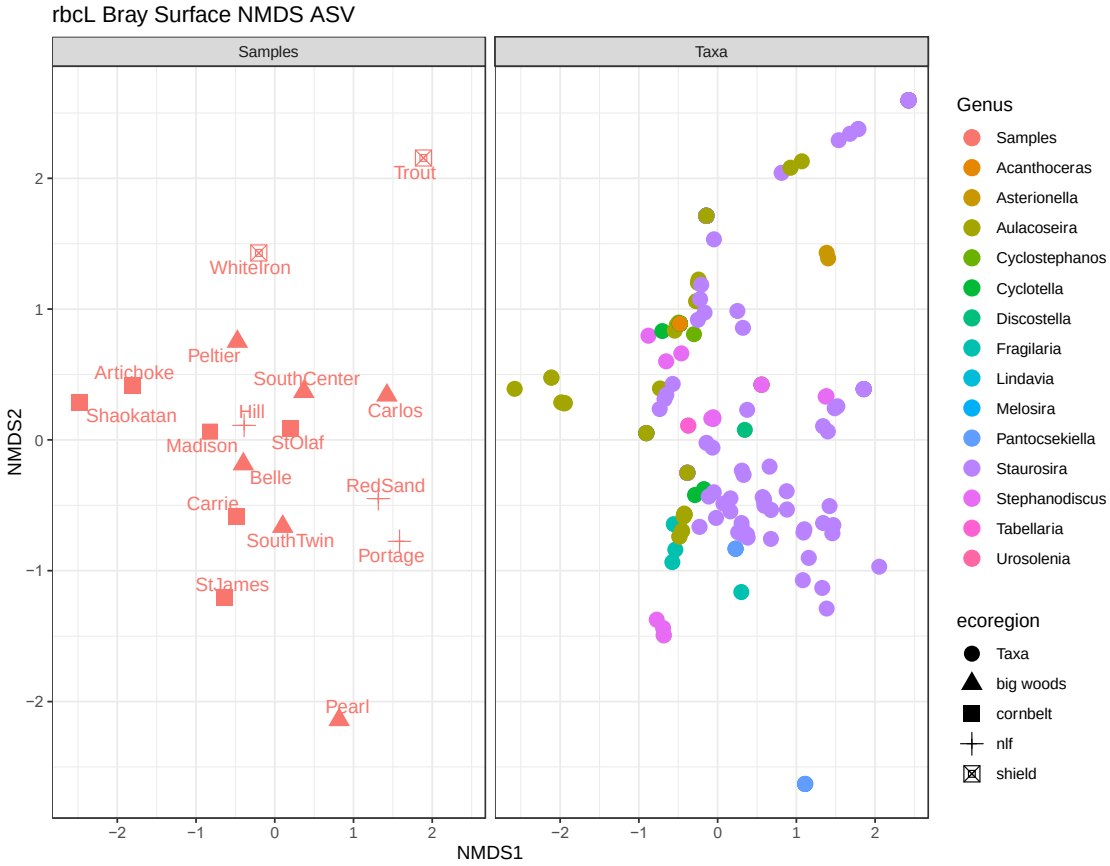


Figure 5.7. Minnesota lakes as samples (left panel), symbolized by ecoregion, and diatom *rbc*L taxa (right panel), colored as genera, arranged in non-metric multidimensional space by diatom amplicon sequence variant relative abundance (Stress= 0.49, $r^2 = 0.9$).

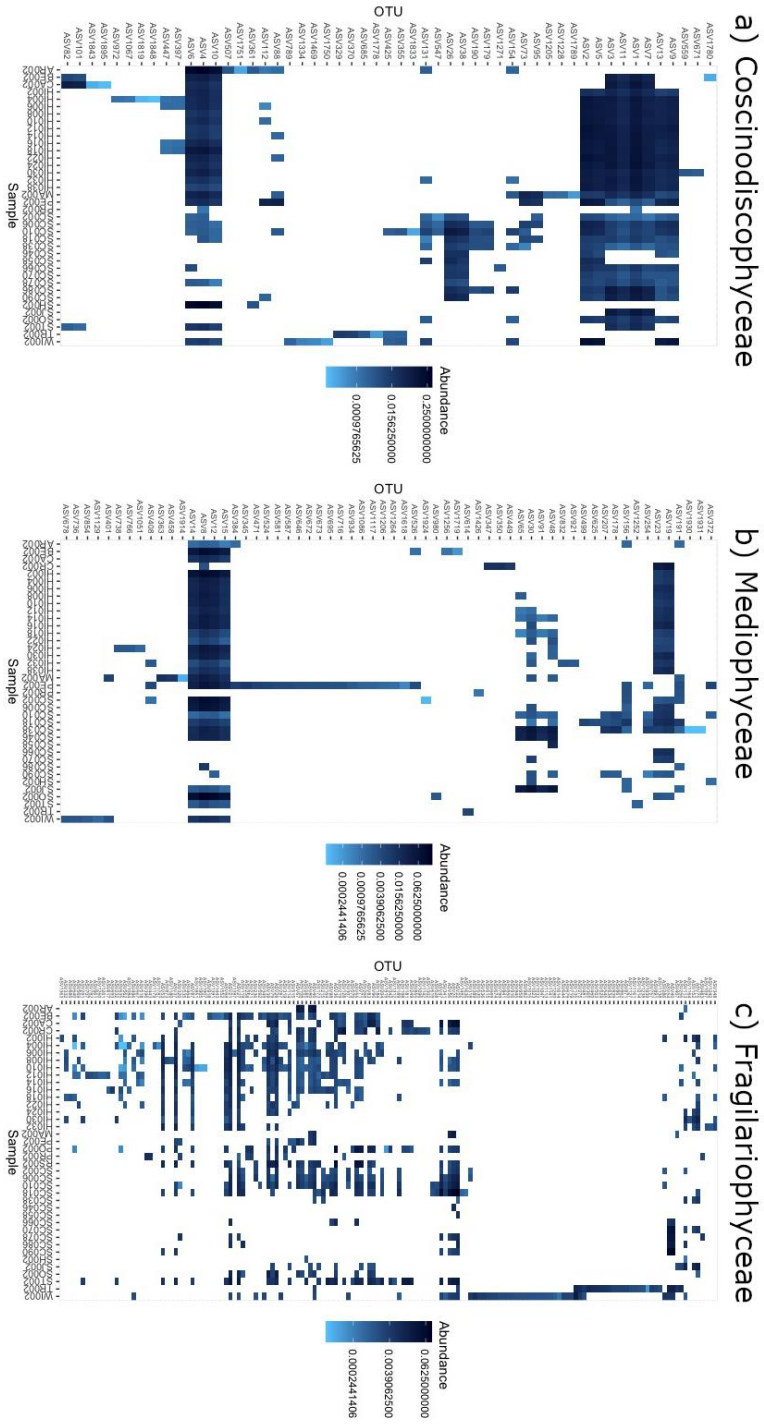


Figure 5.8. Heatmaps of diatom *rbc*L amplicon sequence variant abundance in Minnesota lake sediment samples for a) Coscinodiscophyceae, b) Mediophyceae, and c) Fragilariophyceae. Amplicon sequence variants are arranged by Bray-Curtis distance on the y-axis.

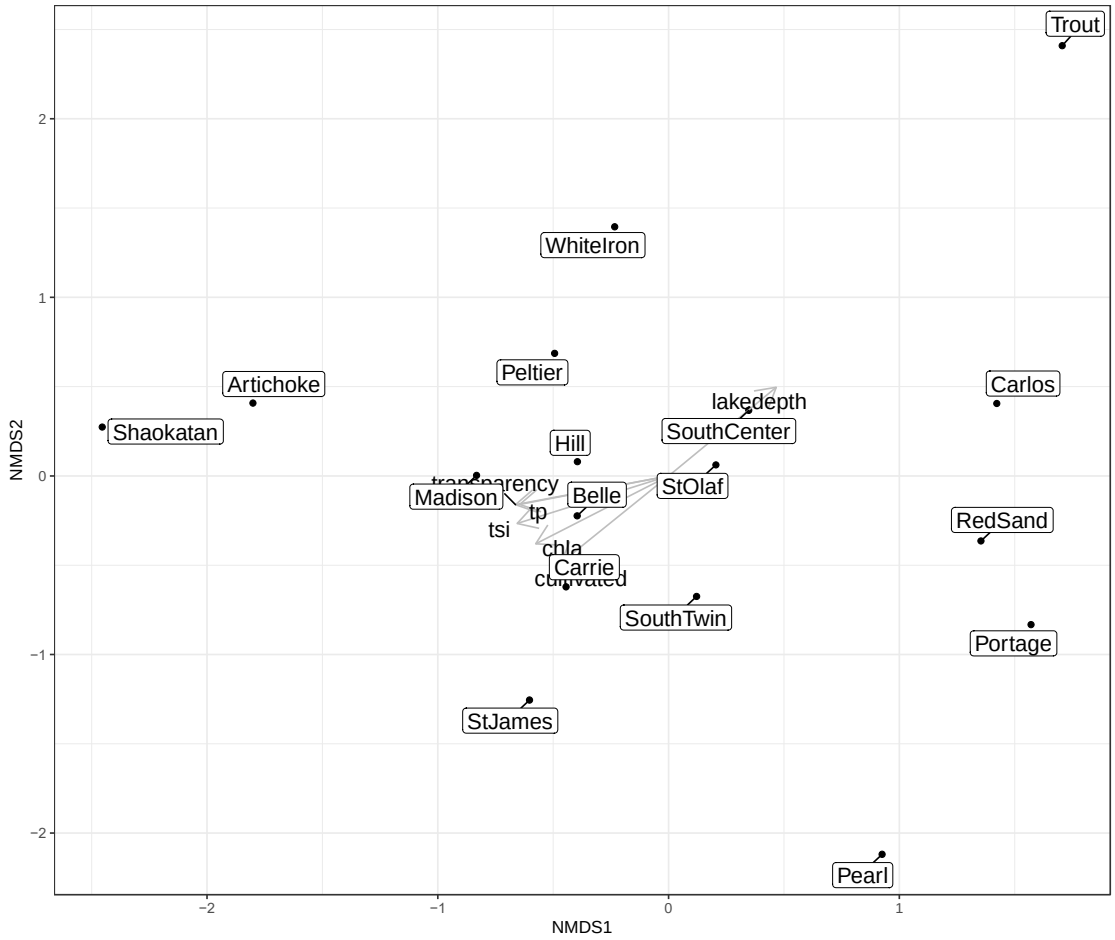


Figure 5.9. Minnesota Lakes arranged in non-metric multidimensional space by diatom *rbc*L amplicon assemblage relative read data overlain with vectors of significant environmental data, including total phosphorus(tp), trophic state index (tsi), chlorophyll-a (chla), lake depth, and the percentage of watershed in cultivated land use.

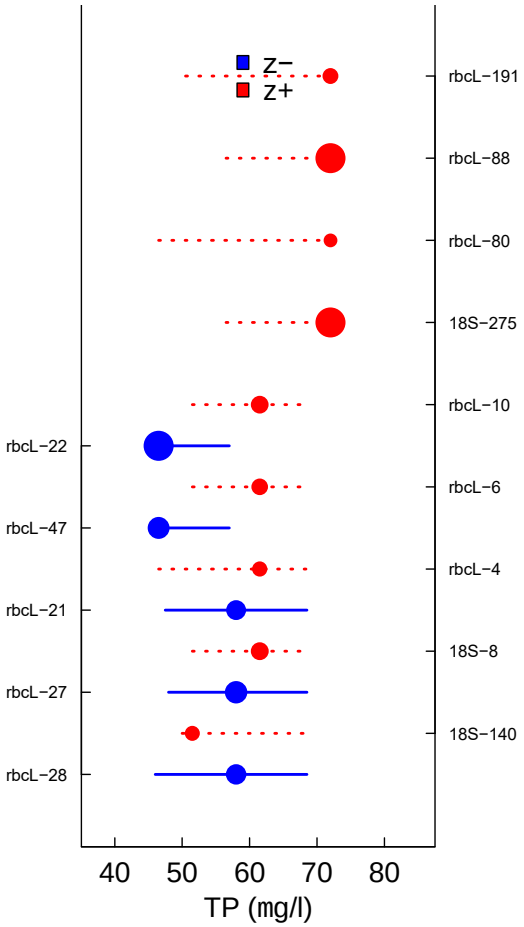


Figure 5.10. Total phosphorus species indicator analysis results for lake surface sediment diatoms detected using amplicon sequencing analyzed with TITAN2. Blue amplicon sequence variants represent low TP taxa whereas red amplicon sequence variants represent high TP taxa.

Figure 5.11. Minnesota lakes as samples (left panel), symbolized by ecoregion, and diatom 18S taxa (right panel), colored as genera, arranged in non-metric multidimensional space by diatom amplicon sequence variant relative abundance (Stress= 0.09, $r^2 = 0.96$).

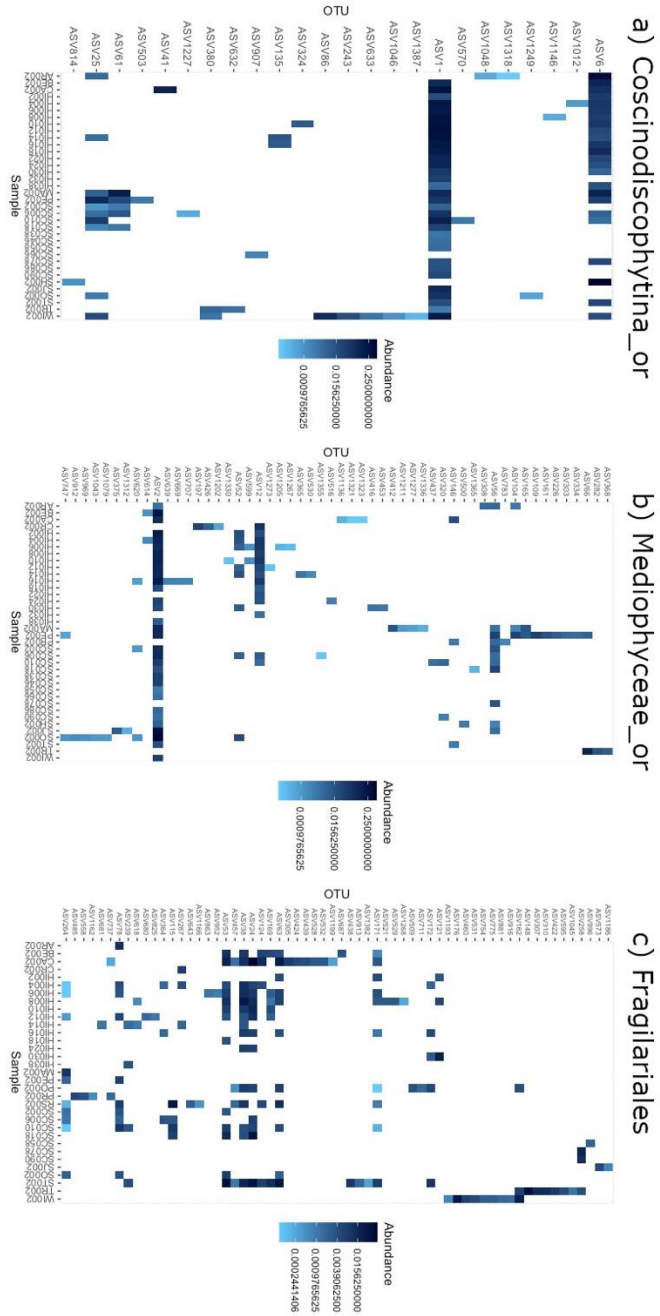


Figure 5.12. Heatmaps of diatom 18S amplicon sequence variant abundance in Minnesota lake sediment samples for a) “Coscinodiscophytina_or”, b) “Mediophyceae_or”, and c) “Fragilariales”. Amplicon sequence variants are arranged by Bray-Curtis distance on the y-axis.

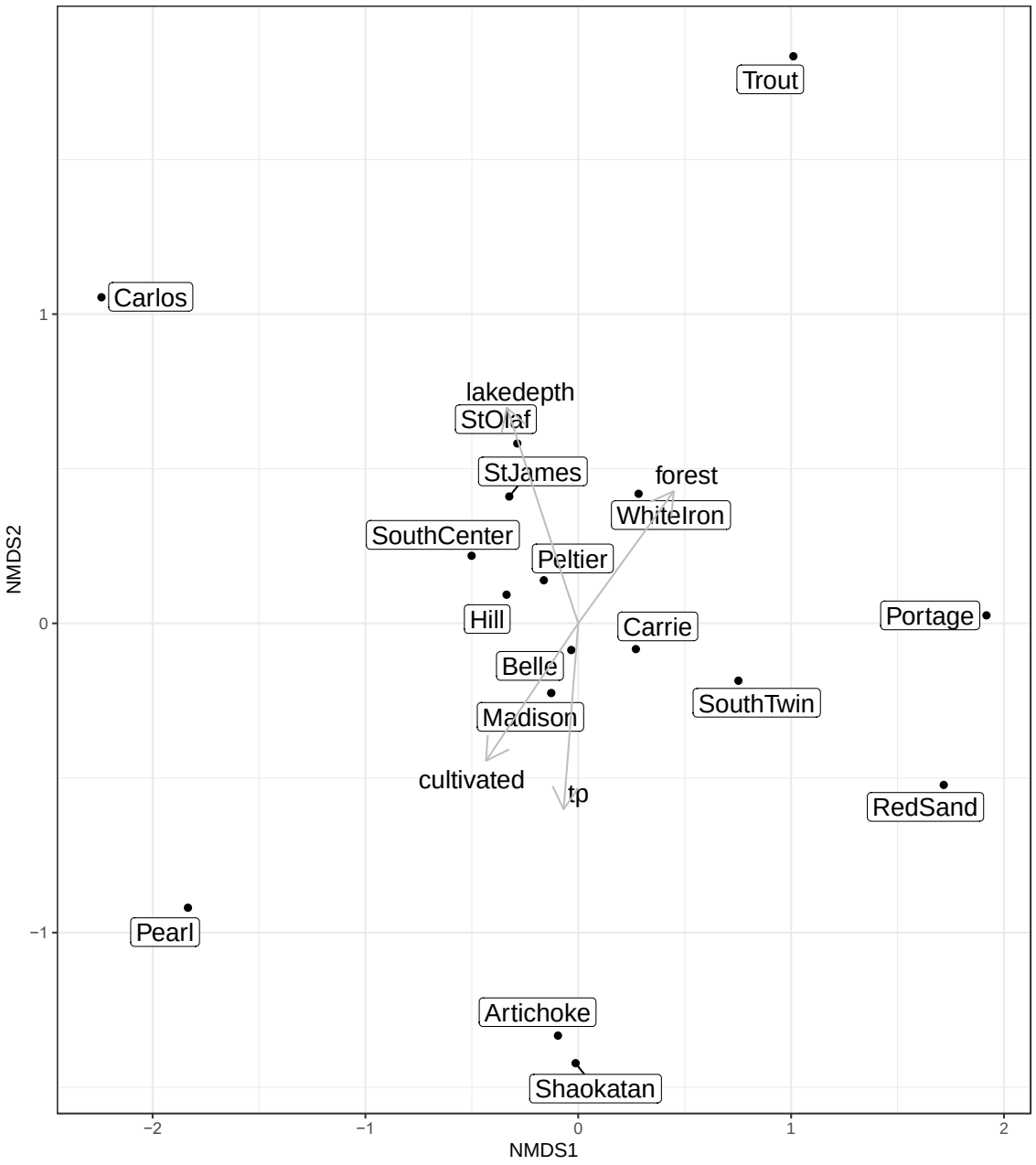


Figure 5.13. Minnesota Lakes arranged in non-metric multidimensional space by diatom 18S amplicon assemblage relative read data overlain with vectors of significant environmental data, including total phosphorus(tp), lake depth, and the percentage of watershed in forested and cultivated land uses.

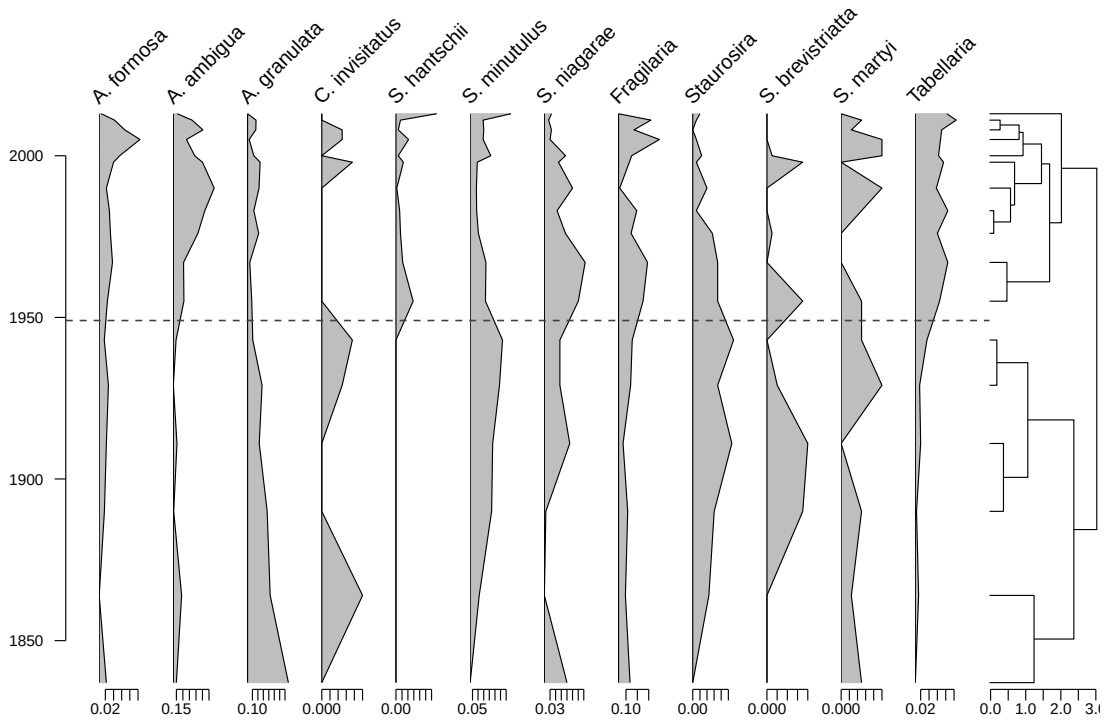


Figure 5.14. The relative abundance of diatoms detected by light microscopy plotted by ^{210}Pb year (C.E.), note the x-axis scale varies in order to illustrate changes each taxon. Taxa plotted here occurred at ≥ 3% abundance in ≥ 3 samples in the Hill Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.

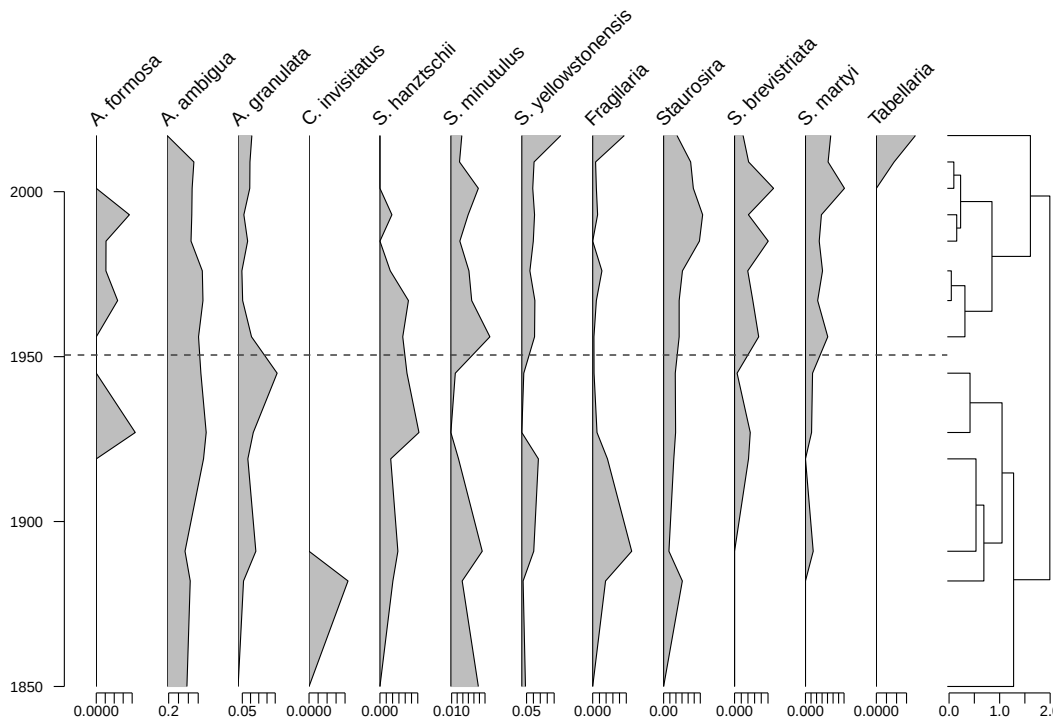


Figure 5.15. The relative abundance of diatoms detected by *rbc*L amplicon sequencing plotted by ^{210}Pb year (C.E.), note the x-axis scale varies in order to illustrate changes each taxon. Diatoms are identified by their sequence matches in the Diat.barcode v7 database; taxa were present at ≥ 3% abundance in ≥ 3 samples in the Hill Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.

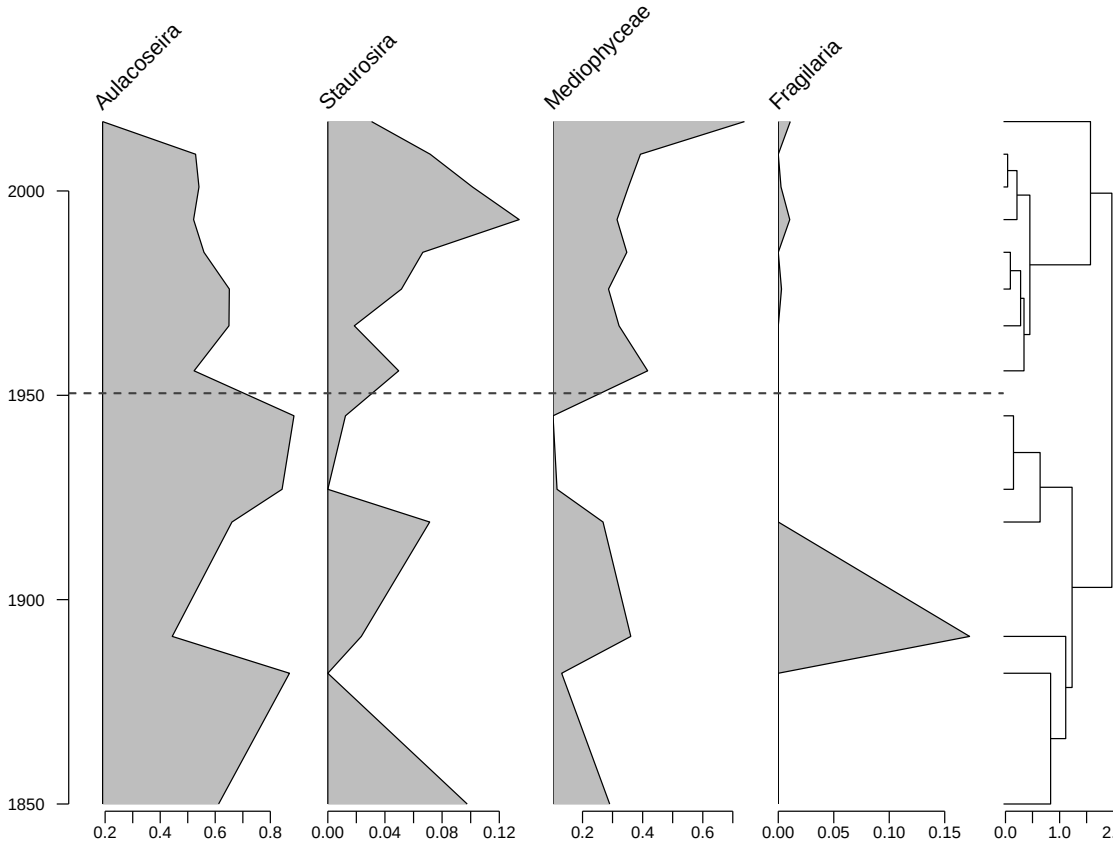


Figure 5.16. The relative abundance of diatoms detected by 18S amplicon sequencing plotted by ^{210}Pb year (C.E.), note the x-axis scale varies in order to illustrate changes each taxon. Diatoms are identified by their sequence matches in the Silva v138 database; taxa were present at ≥ 3% abundance in ≥ 3 samples in the Hill Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.

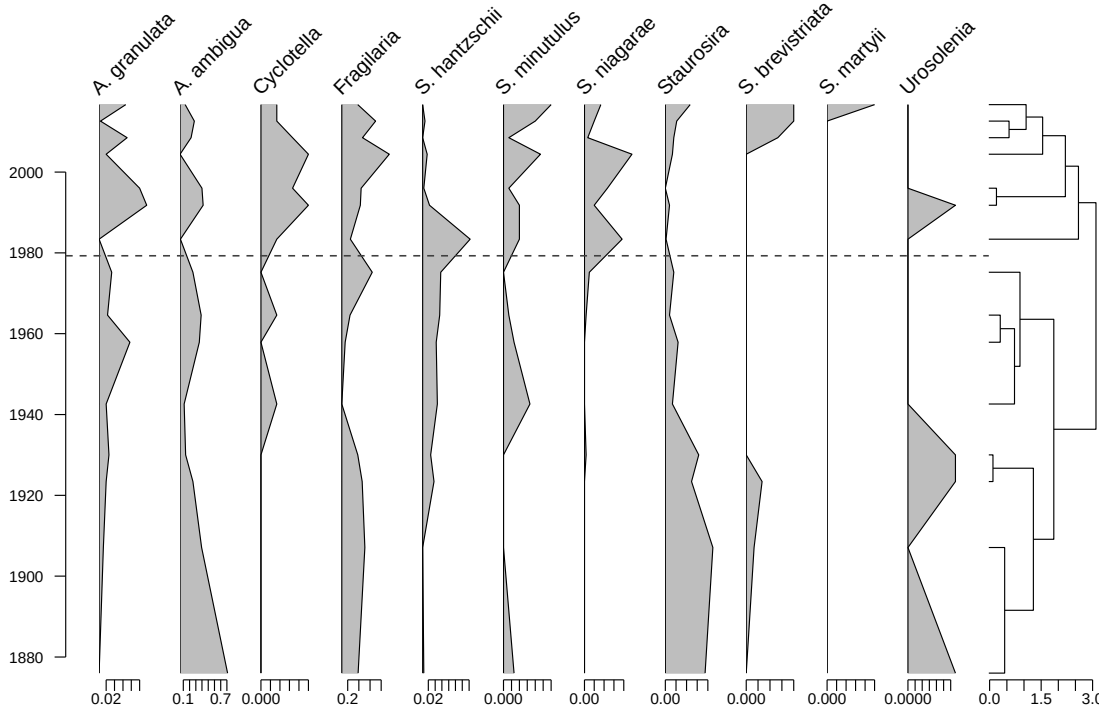


Figure 5.17. The relative abundance of diatoms detected by light microscopy plotted by ^{210}Pb year (C.E.), note the x-axis scale varies in order to illustrate changes each taxon. Taxa plotted here occurred at ≥ 3% abundance in ≥ 3 samples in the South Center Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.

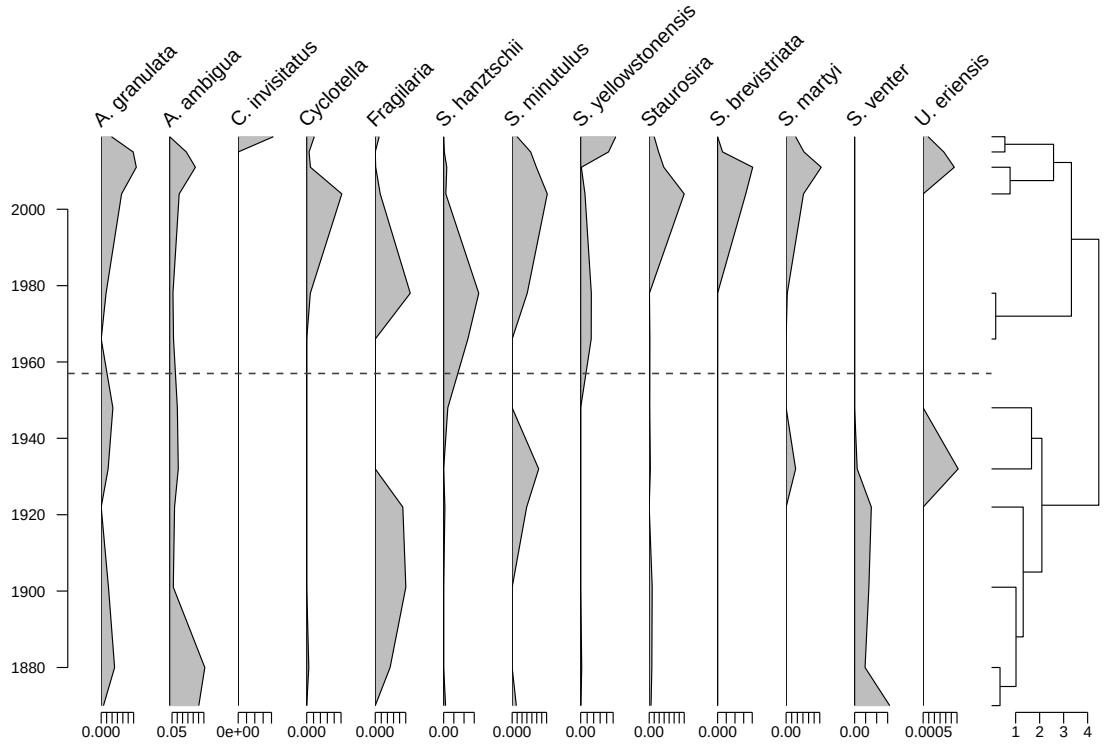


Figure 5.18. The relative abundance of diatoms detected by *rbc*L amplicon sequencing plotted by ^{210}Pb year (C.E.), note the x-axis scale varies in order to illustrate changes each taxon. Diatoms are identified by their sequence matches in the Diat.barcode v7 database; taxa were present at ≥ 3% abundance in ≥ 3 samples in the South Center Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.

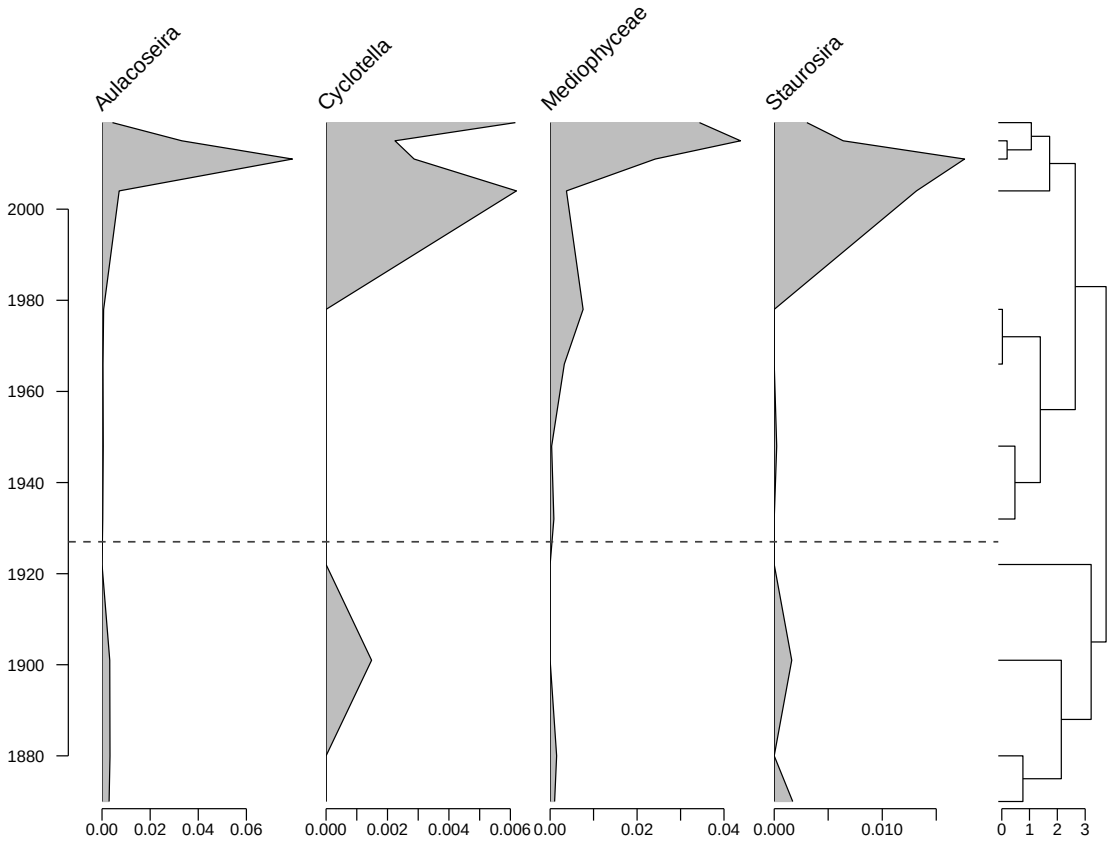
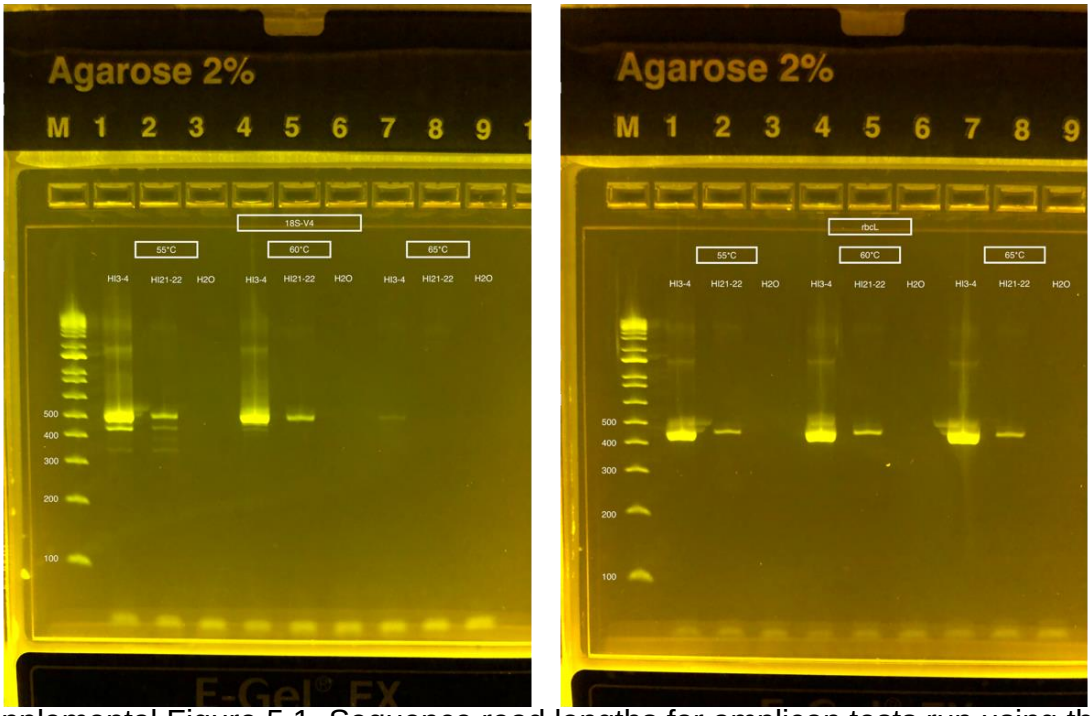
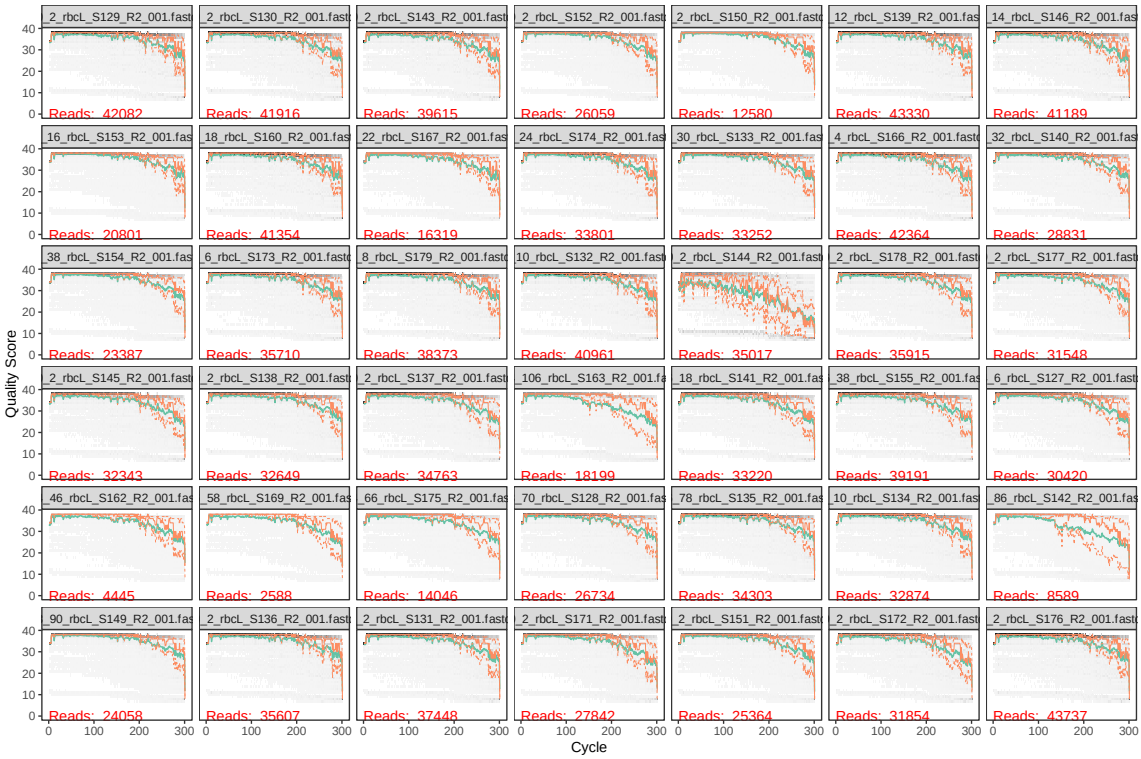


Figure 5.19. The relative abundance of diatoms detected by 18S amplicon sequencing plotted by ^{210}Pb year (C.E.), note the x-axis scale varies in order to illustrate different abundances among taxa. Diatoms are identified by their sequence matches in the Silva v138 database; taxa were present at ≥ 3% abundance in ≥ 3 samples in the South Center Lake sediments. The right panel shows the constrained cluster analysis grouping, with significant breaks represented on the stratigraphy by a dashed line.



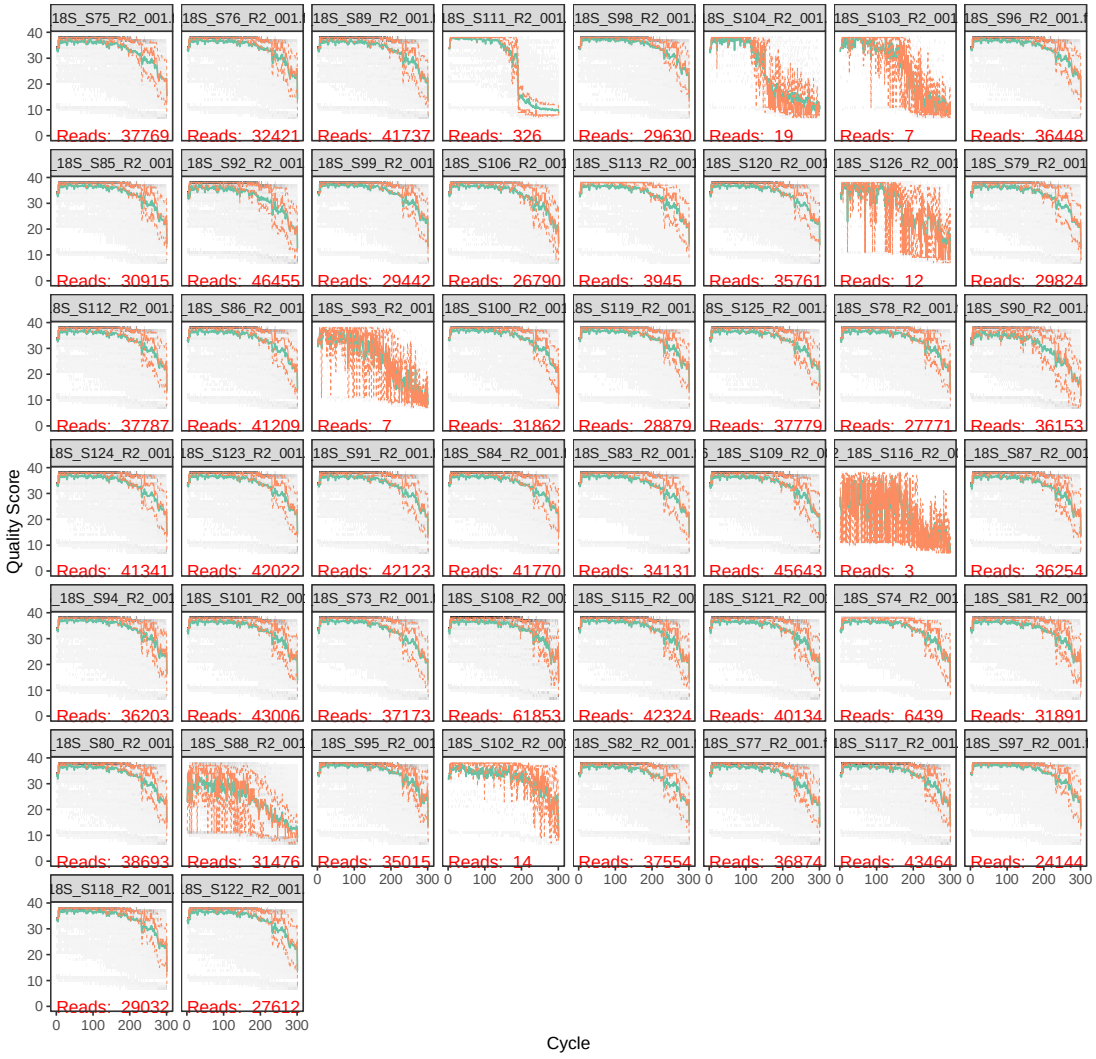
Supplemental Figure 5.1. Sequence read lengths for amplicon tests run using the *rbc*L and 18S primers at annealing temperatures at 55ºC, 60ºC, and 65ºC.

Supplemental Figure 5.2. The *rbc*L forward sequence read Phred quality scores by read position (cycle).

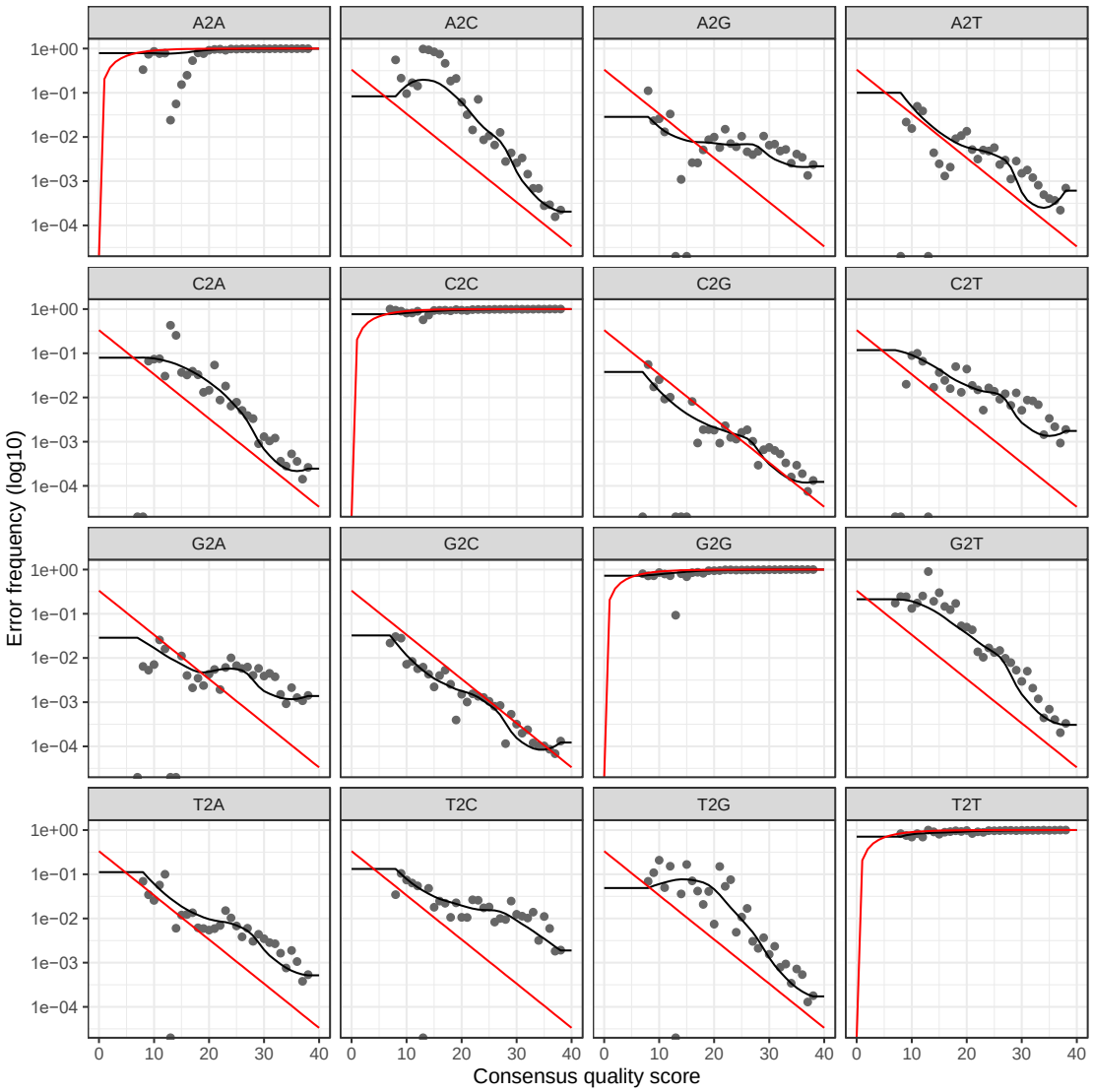


Supplemental Figure 5.3. The *rbc*L reverse sequence read Phred quality scores by read position (cycle).

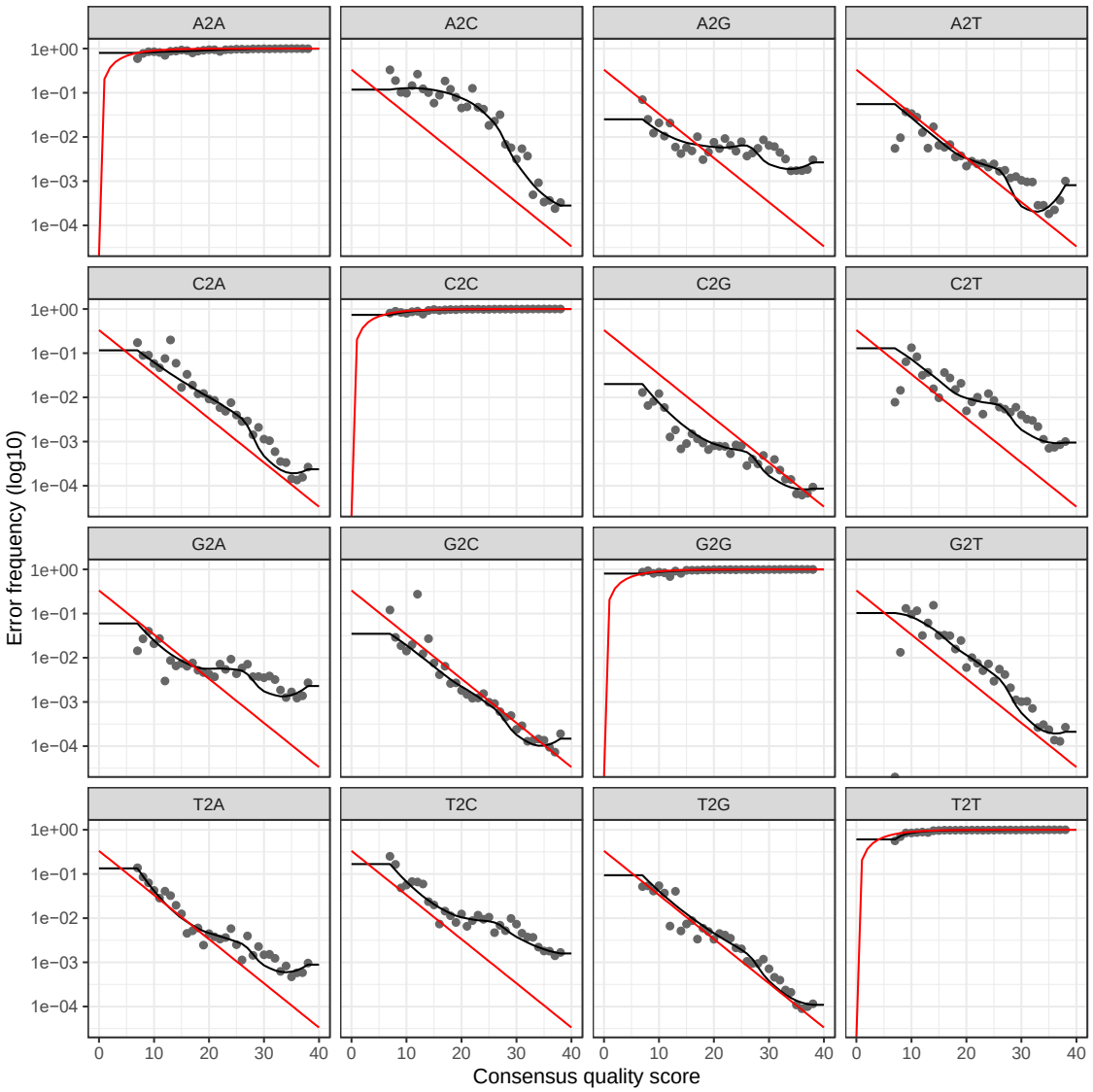
Supplemental Figure 5.4. The 18S forward sequence read Phred quality scores by read position (cycle).



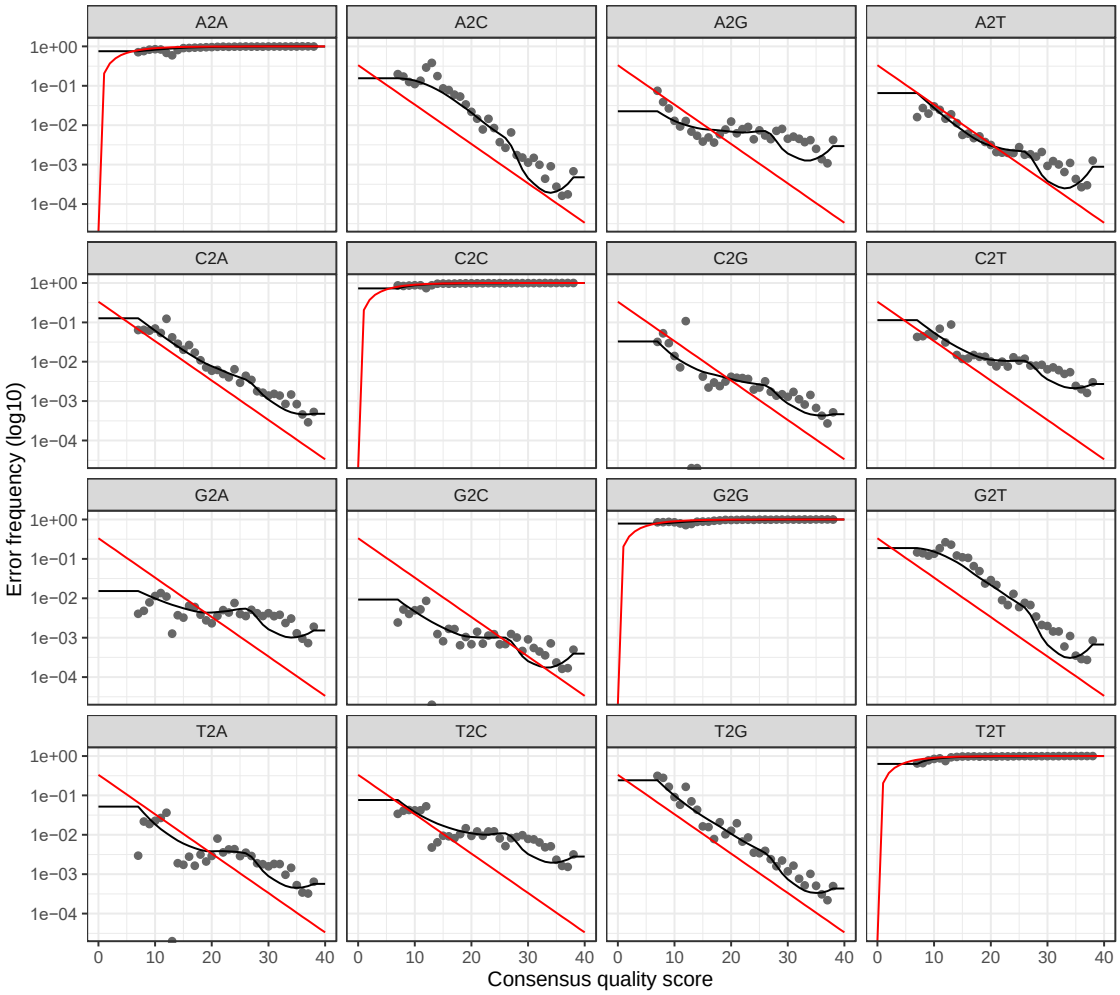
Supplemental Figure 5.5. The 18S reverse sequence read Phred quality scores by read position (cycle).



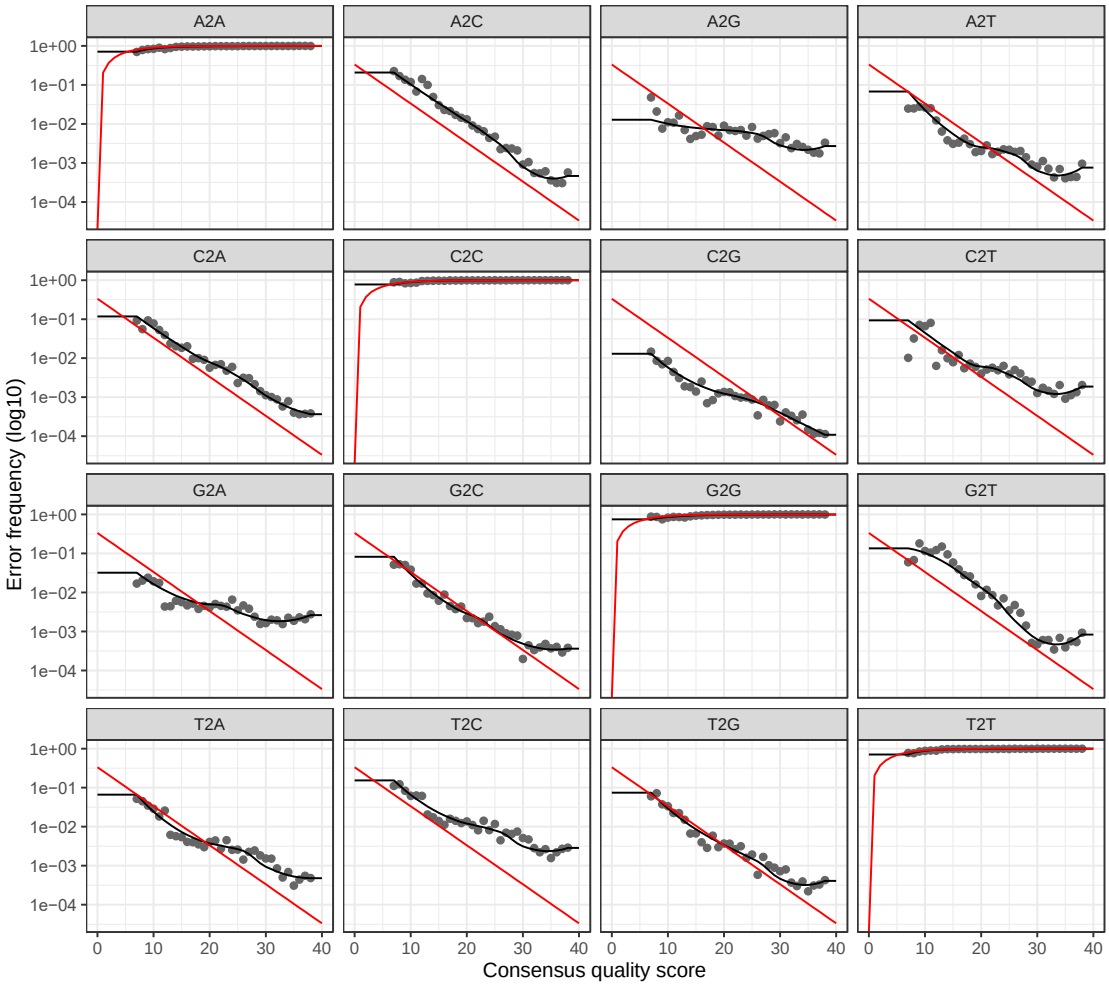
Supplemental Figure 5.6. The *rbc*L forward read error plotted by consensus quality scores.



Supplemental Figure 5.7. The *rbc*L reverse read error plotted by consensus quality scores.



Supplemental Figure 5.8. The 18S forward read error plotted by consensus quality scores.



Supplemental Figure 5.9. The 18S reverse read error plotted by consensus quality scores.

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